MANUAL FOR NUTRITION SURVEYS

INTERDEPARTMENTAL COMMITTEE ON NUTRITION

FOR NATIONAL DEFENSE

MAY 1957

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The Interdepartmental Committee on Nutrition for National Defense is especially indebted to the following consultants who served as the Editorial Committee: Dr. John B. Youmans,[1] Dr. William J. Darby,[1] Dr. William J. McGanity,[1] and Mr. Edwin Bridgforth.

The committee is also greatly indebted to the following, who assisted in the preparation of various chapters in the Manual.

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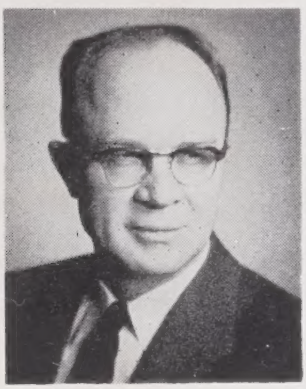
Foreword

DR. HAROLD R. SANDSTEAD, one of the founders and Executive Director of the Interdepartmental Committee on Nutrition for National Defense, lost his life November 1, 1955, in an airline crash while on a mission to speak on nutrition problems in the Near and Far East. He had been a member of the United States Public Health Service since 1934. His experience in conducting nutrition surveys and teaching nutrition extended to all parts of the world. Dr. Sandstead did much pioneering work in developing standardized methods and procedures for nutrition surveys of population groups. The ideals and objectives of this Manual were originated by him. He labored on the outline and many sections of the Manual. It was his hope and is the hope of the Interdepartmental Committee that this Manual will enable not only the collection of nutrition survey data in a uniform manner, but also will facilitate the formulation of practical measures to improve the nutritional status of undernourished people.



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Introduction

The "first provisional edition" of the Manual was issued January 1956, as a guide for conducting nutrition surveys of Armed Forces in Near and Far East countries. It was used in field tests in four countries in 1956 and 1957, namely, Iran, Pakistan, Philippines and Korea. These field tests led to various changes in the Manual, and the committee recognizes that additional improvements will be needed from time to time.

I. Objectives

The principal objective of this Manual is to serve as a guide for survey teams in collecting data for an assessment or appraisal of the nutritional status of a population. To accomplish this objective, it is anticipated that the Manual will assist the survey team as follows:

(1) To establish uniformity in methods, techniques, and procedures for conducting a nutrition survey, thus enabling a reliable comparison of the results of surveys within and among countries. It is realized that some more refined methods might be valuable. However, of necessity, only relatively simple, well-tested methods adapted for field work can be used.

(2) To serve as a reference to insure maximum coverage of the major facts considered essential in appraising nutritional status and to permit practical, effective recommendations to correct any nutritional deficiencies that may be found.

(3) To serve as a guide for the interpretation of the dietary, biochemical, and clinical data collected.

(4) To serve as a guide in defining the responsibilities and duties of various team members.

(5) To assist in training local personnel to conduct nutrition surveys and in establishing a permanent nutrition service within the Armed Forces.

It should be recognized that this Manual is designed primarily for surveys of military forces. However, the basic approach and methodology is the same in surveying civilian populations, and even in surveys restricted to military forces it is necessary to study many phases of civilian food supply, population characteristics, health, and economic conditions.

II. Interdepartmental Committee on Nutrition for National Defense

The Interdepartmental Committee on Nutrition for National Defense was organized for the primary purpose of appraising and improving the nutritional status of populations in nations friendly to the United States. The committee is composed of representatives from the Departments of Defense, Army, Navy, Air Force, Agriculture, State, and Health, Education, and Welfare, the International Cooperation Administration, and the Atomic Energy Commission. The committee serves as a clearing center for the collection and appraisal of nutrition data, initiation of field

surveys and programs, and recommends corrective measures. A secretariat, located at the National Institutes of Health, Bethesda, Md., has been organized to serve the committee. A group of outstanding specialists in nutrition and agriculture with experience in foreign areas serve as consultants to the committee.

III. Means of Assessment of the Nutritional Status of a Population

In appraising the nutritional status of a population it is essential that many economic, sociologic, and related problems be considered in addition to data on nutrition and health. The various manifestations of undernutrition and malnutrition vary considerably in degree and type in different countries and may not always be predicted. More needs to be known than mere food production figures, for in evaluating nutritional status, the important factors are the quantity and quality of food actually consumed, considered in relation to "requirements" or need. Estimates or even fairly accurate data on average caloric and nutrient intake are not sufficient for accurate evaluation of nutritional status, since inequities in distribution, availability of nutrients in food and many other factors may influence the situation. The consumption of a diet that is marginal in protective nutrients may permit survival for an indefinite period. However, such populations usually have a low life expectancy, increased disease rates, lowered physical and mental efficiency, and other manifestations of ill health.

The chapters which follow will present in some detail the various procedures and methods necessary to assess and evaluate nutritional status.

IV. Operating Procedures

1. Organization of Survey Teams

The Field Survey Director has overall supervision of the survey teams. In addition, a survey team director will be appointed for each country, who will direct and coordinate the activities of all survey team members, and serve as liaison to the United States Embassy, United States Operations Missions, Military Advisory Assistance Groups, and the host Government heads. In general, a survey team will be composed of the following specialists: physician, biochemist (laboratory director), two or more laboratory assistants, food service and nutrition specialist, food technologist, and agricultural economist. In addition, it is expected that the host Government will assign personnel to work with the survey team to receive training and experience in nutrition surveys. Members who will be serving in more than one country are responsible to the team director of the country in which they are working.

In general, the various chapters of this Manual define the responsibilities and activities of the team members; however, to avoid omission of pertinent data and duplication of effort, consultation and briefing of the team by the survey director will be necessary. The team leader shall have responsibility for general supervision of the team and shall coordinate the programs of work, including assignments to obtain supplemental information such as is outlined in chapter 9.

2. Proposed Work Schedule

In the detailed discussions of following chapters there are outlined procedures that permit an adequate sampling of individuals and provide a broad basis for evaluation of their nutritional status. In the 60-90

operating days of the survey, the principal activity will be the collection of clinical, biochemical, and dietary evidence of nutritional status from a large representative sample of individuals in the Armed Forces. Ancillary data needed for the interpretation of this evidence will be obtained.

Upon arrival in the country, initial organization and scheduling of activities by the members of the team for the period of stay will enable completion of the survey objectives in the time available. Such scheduling will of course be flexible, and should recognize the probabilities of delays due to weather, uncertainties of travel, and the like.

Ancillary work assignments should include:

(1) Visits to scientific and educational institutions for the collection of data on research and teaching programs related to the country's nutrition.

(2) Visits to hospitals for the collection of data on level of medical care, general disease, nutritional debility, food service procedures.

(3) Visits to service and training schools, particularly Quartermaster and Medical.

(4) Performance of functional and therapeutic tests.

(5) Visits with physicians and other medical educators in the country.

(6) Evaluation of such influences as malaria, amoebae or other parasites on nutritional status.

3. Protocol

Upon arrival in a host country to undertake or participate in nutrition surveys, the director of the survey team should report to the appropriate United States officials of the Department of State and the Department of Defense. Each member of the survey team should register with the American Embassy. Such courtesy calls within the country concerned should be arranged by the American Embassy or United States Military Group. When leaving the country, the survey director should report to appropriate officials.

4. Reports

a. Reporting Through Channels

Consultants to the Interdepartmental Committee on Nutrition for National Defense serve in an advisory capacity. Findings obtained during a survey may be discussed with discretion on a "need-to-know" basis, but recommendations and written opinion, except as specifically authorized, shall be transmitted only to the chairman and executive director of the Interdepartmental Committee on Nutrition for National Defense.

Policy decisions may involve various international problems. If a consultant or other team member bypasses established channels and organizational echelons, it may prove embarrassing and may delay or prevent the accomplishment of the mission.

A tentative preliminary report may be made to appropriate United States officials before leaving a given country, as circumstances indicate. It should be recognized that many of the facts gathered by the survey team may not be fully analyzed and evaluated at the time the team leaves the country.

b. Written Reports

Interim reports shall be transmitted to the executive director as requested. A comprehensive team report shall be completed promptly and transmitted to the executive director.

c. Rights to Publication

The information obtained in nutritional surveys conducted for the Interdepartmental Committee on Nutrition for National Defense shall be the property of said committee. Such information shall not be made available to the press nor published by an individual or private group except with the written permission of the chairman or executive director of the Interdepartmental Committee on Nutrition for National Defense.

d. Informing the Secretariat

The secretariat, among other things, serves as a clearing house for information and as custodian of the committee's records. Consequently, it is essential that copies of all relevant correspondence and reports, as well as readily available documents relating to the work of the committee, be transmitted to the secretariat.

Sampling

This chapter will describe basic principles involved in the use of samples, for the estimation of the characteristics of populations. Sampling for clinical and biochemical evaluation of the Armed Forces surveyed, is discussed. (The plans for dietary evaluation are given in chapter 5.) Suggestions are advanced as to how the sampling should be done by the survey team, but no detailed catalog of procedures is given. A sampling plan which is appropriate for country A, may not be so for country B, due to differences in general organization of forces, location and size of troop bases, facilities for transportation between bases, etc. If, however, the team adheres reasonably closely to the basic principles given, a sufficiently representative sample of the population should be obtained without serious difficulty.

I. Objectives of Sampling

It has long been the experience of workers in many fields, that neither time nor money exists for examining the whole of every population about which information is desired. For instance, a farmer wishing to know the quality of his seed for planting, will test only a small proportion of his seed supply as a sample by which germination rate for the whole supply can be estimated. Sampling investigations can provide *accurate* and *precise* information about a large population by the examination of only a portion of that population.

Accuracy of an estimate depends upon the unbiased sampling of the true or population values. In other words, the methods of measurement or classification must be correct. For example, the determinations of hemoglobin are made with instruments standardized so that a value of say 12.2 gm/100 ml for hemoglobin would be reproducible by any other investigator, or a finding of edema in an individual would be classified likewise by any other qualified examiner. The problems of accuracy in an investigation are general ones, whether or not sampling is involved. The errors in results so introduced are usually called *nonsampling errors*, to distinguish them from the *sampling errors* which are due to the inherent variation of sample estimates about the population values. The nonsampling error is unaltered by change in the sample size, whereas the sampling error can be reduced by enlarging the sample, but only in proportion to $\sqrt{n}$, so that quadrupling the size only halves the sampling error. (n = sample size.)

Precision of an estimate refers to its reproducibility or the closeness of estimates obtained if similar samples are taken. The true population values for the characteristics studied in an investigation can be estimated as closely as desired by taking a sample sufficiently large to meet the specifications of precision needed. For example, if the finding of any condition in the population studied is to be compared with a "normally expected" 1 percent rate of findings, then, if the actual rate of occurrence of this condition in the studied population is 3 percent, a properly drawn sample of 500 individuals would be more than adequate to establish the

difference. This statement will hold no matter how large the studied population is, be it 50,000 or 50,000,000.

II. The Population and its Stratification

The population to be surveyed is the Armed Forces of the country, at the time of the survey. The overall objective is to obtain, by the examination of a representative sample of individuals, estimates of nutritional status of the population.

It may be that this status is very different for some portions of the population than for others (e.g., Army compared to Navy), and it may be desirable to define certain subpopulations (or strata), such as these, from which samples of sufficient size will be drawn to allow adequate estimation of their nutriture as a separate group, in addition to their inclusion in the total population estimates.

It seems likely that each branch of service of the Armed Forces, (Army, Navy, Marines, Air Force), should be considered as a separate subpopulation for selection of survey samples. Specification of additional subgroups within each Service will probably be appropriate, also.

One such subdivision might be: (1) new inductees; (2) trainees, at completion of basic training; (3) trained troops, on active duty. The trained troops might be further divided according to type of duty, e.g., Tank, Artillery, Infantry Rifle Company, Quartermaster, etc.

Another subdivision might be according to location or other characteristics of the bases where the troops are stationed. Suppose bases are located in coastal, desert, and mountain areas. Food supply, both locally obtained, and available by transportation facilities, may be very different.

An alternative subdivision by bases would be appropriate if it were found that there are regional food purchase centers for the Armed Forces, each supplying food to bases in its vicinity, but with possible or probable differences in type and quality of food supplied by different centers. Bases supplied by each center would be considered as a stratum of the population, from which samples would be drawn.

Such attributes of the individuals in the population as: (1) age; (2) length of service; and (3) preinduction residence and/or occupation, are also relevant in assessment of nutriture. These, however, cannot be a basis for stratifying the population preparatory to sampling, as it is unlikely that men will be grouped together in units based on any one of these characteristics. It will be sufficient to record such information on the individual records of sampled individuals, for later analysis of their relevance.

If feasible for the country surveyed, economic status might be indirectly estimated by a recording of preservice occupation of the soldier or of his parents, e.g., on a broad basis such as farm owner, farm laborer, miner, urban small business owner, industrial unskilled worker, etc. Specific categories would be set up in advance of the survey, tailored to fit the more usual occupations of the country which might identify groups differing in nutritional status.

Other ancillary data such as sunshine, temperature and other physical characteristics of troop bases should also be recorded at the time of the survey. These factors can then be taken into account in the analysis of the survey data.

III. Probability Sampling

To obtain true representation of the population, any sample of individuals from the population must be selected *at random*. Every individual must have a chance of inclusion in the sample. These chances do not necessarily have to be equal. For instance, if the Navy is small, but an estimate of nutritional status of the country's sailors is desired, 10 percent of all Navy men might be examined, while only 5 percent of all Army men might be examined. This *disproportionate sampling* in different strata will of course have to be allowed for in making estimates on the total population.

The essential point in *probability sampling* is that individuals selected must be chosen at random from all those in the stratum being sampled. As a simplified illustration, suppose that from a group of 10 men, 5 were to be chosen for examination. Their names (or corresponding numbers) could be written on slips or tags, put in a hat, thoroughly mixed and then five of the slips removed blindfolded, to give the randomly selected sample. If however, the total group consisted of 1,000 men, and again five were to be chosen for examination, this system would be cumbersome; for such cases, tables of "random numbers" are commonly used. Each man is assigned a number, and the men for the sample are chosen by picking five of their numbers at random, using the tables. For illustration, the following row of digits is taken from one such table: [1]

64978 17343 77305 91360 22086 38928 65507 18258 17231

If the table is entered haphazardly and this line chosen to be the basis for the above sample selection, then using the first three digits in each sequence of five, men numbered 649, 173, 773, 913 and 220, would be chosen.[2]

As a general procedure, the use of this method of selection is generally safest in assuring randomness of choice, even when drawing from a hat seems easier, as it is often doubtful if mixing of slips of paper in hats is sufficiently thorough to assure randomness.

Minor departures from the principles of random selection of the sample from the population may be tolerable, but major departures may well lead to evaluation of subjects atypical of the total population. Study of specially selected unusual individuals and groups may be of interest, but for a representation of the population, random choice is necessary.

The discussion thus far has considered the individual as the basic "element", (or *unit*), of the sampling. For large populations such as the Armed Forces of a country, *direct selection* of individuals at random is neither feasible nor necessary.

The most efficient choice of size of sampling unit—to obtain the most information for given amount of time for surveying—depends on the variability in the nutritional status of individuals in different organizational units of the forces. As an extreme situation, line-duty troops might be predominantly long-service men, with messes of battalion size and large differences in nutritional adequacy of diets by battalion. In such a case, it would be most desirable to sample as many battalions as possible,

[1] The Rand Corp., *A Million Random Digits with 100,000 Normal Deviates*. Glencoe, Ill., The Free Press, 1955.

[2] Tables of random numbers, with further example of how to use them, are given in A. Bradford Hill, *Principles of Medical Statistics*, 6th edition revised and enlarged, pp. 288-306.

examining only a few men from each, since the greater variability in nutriture will be found *between* battalions, compared to that between men *within* battalions. With incomplete knowledge, in advance of the findings of the survey, as to what the actual situation is, it will be desirable to "spread" the sampling as broadly as possible over all the forces, consonant with possible difficulties of travel between locations.

IV. Sampling Procedures

Before any sample selection of subjects for examination can be made, information must be obtained to enable appropriate stratification. The following example describes in outline, a satisfactory approach.

Suppose the Armed Forces are found to be composed approximately as follows:

Army	120,000
Marines	10,000
Navy	5,000
Air Force	5,000
Total	140,000

Additional subgroups can be defined for each Service, with approximate numbers in each:

Army.—20,000 in training; 40,000 at coastal bases; 30,000 at mountain bases; 20,000 at desert bases; 10,000 at isolated border posts. Training is for 6 months after induction, at two large central bases, each with about 1,500 new men accepted monthly. The two bases draw men from different sections of the country; both are readily accessible. There are five divisions of men at the various coastal bases, four divisions at the various mountain bases, and three at the various desert bases. Some of the divisions are under-strength in their numbers, but differences are not marked. Each base consists of an Army division, except for two divisions which have men stationed at several neighboring bases. The men at isolated border posts are stationed in groups of sizes 5 to 500, but usually 20 to 30.

Marines.—10,000 men, stationed at two similar seacoast bases of approximately equal size.

Navy.—5,000 men, located at three shore stations (with approximately 1,000 men at one, 500 at each of the other two), and the other 3,000 on some 20 ships of varying sizes.

Air Force.—5,000 men, stationed at some 10 bases scattered around the country, two bases being large, the others small.

To sample from these strata, one might proceed under ideal conditions as outlined below:

Army.—For the men in training, visit both bases. At each, select at random, one-fourth of the companies of men in their first month and men in their last month of training (375 men from each of these two groups at each base; total of 1,500 men).

From the five coastal divisions, select two divisions to be sampled at random.

From the four mountain divisions, select one division to be sampled at random.

From the three desert divisions, select one division to be sampled at random.

For the border posts, allot one week of the survey for sampling them. Obtain further detailed information as to locations, and select on a *judgment* basis, a schedule for visiting enough of these posts to examine 250 to 500 subjects and to investigate their feeding. Posts visited should be sufficiently scattered to give a representative picture of all posts, but will be chosen with regard to fitting a travel schedule.

Marines.—From the two bases, select one base to be sampled at random.

Navy and Air Force.—Allot one week of the survey for sampling these two groups. For the Navy, select one shore station at random, and by random procedures, pick 375 men for examination. From ships in accessible ports, make random choices of enough to provide 375 men for examination. For the Air Force, select at random, one of the two large bases and two of the eight small bases. From these bases choose 750 men for examination.

From each Army division, and from the Marines, 1,500 men will be selected for the "main sample." The basic unit of sampling will be the company. The companies, or equivalent groups of men of number approximately the same as in a company, can be listed for each division, and a sufficient number selected at random. Companies may alternatively be chosen, randomly, from a further stratification of the division according to its Table-of-Organization, giving the number of each type of company it contains, e.g., Engineer, Quartermaster, etc.

An important point to be made here is that whenever a given company is chosen, every man in the company must be included in the "main sample." This includes men on sick call and men in the hospital. Special arrangements may have to be made for the examination of some of these men. It may also become impossible to examine others, but every effort should be made to include all men in the company, since serious biases may result from examination of only those well enough to get to the examination area.

The processing of the men selected for examination is described in the following sections.

V. Total Sample Sizes

As the preceding sections imply, the total size of sample needed to accomplish the objectives of the survey cannot be *exactly* specified in advance. There must be a balancing of the facilities of the team and its problems of travel to the locations where men for sampling are stationed, against estimations of sample sizes needed to evaluate nutriture for both the total population and for appropriate strata within the population.

Biochemical facilities could not be sufficiently extensive to allow blood and urine testing of all individuals examined clinically. On the other hand, it will be desirable to obtain sufficient height-weight-age data to enable the preparation of "internal standards" of weight status, and to enable very detailed evaluation of different units of the Forces according to differences in body weight, adjusted for age and height.

Further, for some of the clinical signs of possible nutritional inadequacy, especially those which may be expected to be infrequent, a much larger total sample of individuals is needed to obtain sufficiently precise estimates of prevalence of the signs in the population, than would be needed for findings of higher frequency. For example, if a certain

nutritional deficiency sign prevails in 0.5 percent of the troops, the examination of say, 10,000 troops will reveal about 50 cases, whereas with 2,000 troops examined, only about 10 cases would be found. The estimate of prevalence, given 50 cases found in 10,000, would be 0.50 ± 0.07 percent, whereas with 10 cases in 2,000, it would be 0.50 ± 0.16 percent. The former estimate should be adequately precise; the latter is questionably so.

Due to these principles, the clinical sampling will be in two phases. The "main sample" of men selected will be divided into two groups with four of every five men receiving an *abbreviated* clinical examination while every fifth man, chosen systematically, will be sent for a *detailed* clinical examination. There, again systematically, every fifth man will have a blood sample drawn and will be scheduled to give a fasting urine sample. (This means that every 25th man from the "main sample" is selected for the biochemical sample.)

The method of systematic sampling with a random start is used in allocating men to the abbreviated or detailed clinical examination. Start with a complete list of the men in the "main sample." (This list will include those on sick call and in the hospital. Those on sick call or in the hospital, should be listed in their normal position on the roster, not at the end.) The men in the list are then numbered consecutively. Now, a number between 1 and 5 inclusive is drawn at random. This number determines the number of the man to receive the first detailed clinical examination. Every fifth man thereafter will receive a detailed clinical examination while all others will receive the abbreviated examination. Thus, if the number drawn were 3, men whose numbers were 3, 8, 13, 18, etc., would receive the detailed examination.

The men who are to receive the biochemical examination are chosen in the same way from among those receiving the detailed examination. Here a second number between 1 and 5 inclusive, is drawn. If the number drawn were 4, this would mean that the fourth man to receive the detailed clinical examination would also receive a biochemical examination. This would also apply to every fifth man thereafter from the detailed examination. In the example with the first number 3 and the second number 4, men numbered 3, 8, 13, 18, 23, 28, 33, 38, 43, 48, 53, 58, 63, 68, 73, 78, etc., would have a detailed examination while men numbered 18, 43, 68, etc., would also have a biochemical examination.

The following total sample sizes are not *targets* for each team in each country. They are presented as an illustration, suggested as reasonable to attain with the given time and facilities, and probably sufficient in size for the evaluations of nutriture. The assumption is made that the examination facilities as described in chapter 3, will process 500 to 600 individuals of the "main sample" in a full day of examinations, and that the equivalent of 25 full days of examinations is attained.

Total subjects in the "main sample"	12,500 to 15,000
Subjects given abbreviated clinical examination	10,000 to 12,000
Subjects given detailed clinical examination	2,500 to 3,000
Subjects given biochemical samples	500 to 600

VI. Systematic Subsampling Within the "Main Sample"

The preceding section indicates the plan for selecting one-fifth of the subjects in the "main sample" for a more detailed clinical examination than the other four-fifths receive, and selecting one twenty-fifth of the subjects for biochemical sampling. These proportions are suggested as

probably satisfactory, but can be modified in the field at any time to procure a smoothly flowing examination "production line," or to meet changes in number of troops available for survey. Thus, in planning a survey, it might be thought that 500 to 600 men could be given abbreviated examinations, 125 to 150 men could be given more detailed examinations and 25 to 30 biochemical samplings could be run. This would give the sampling ratios of 1 to 5 and 1 to 25 as previously discussed.

If, on the other hand, it was found that only 375 to 450 men could be handled by the group giving abbreviated physicals, it would be necessary to use a ratio of 1 to 4 for detailed physicals and 1 to 20 for biochemical examinations. Another change which might occur would happen if a base chosen for survey only contained 250 men. Here, the "main sample" will be all of the men at the base and 1 out of every 2 men would be given detailed examinations, and 1 out of every 10 would be taken for biochemical tests.

It may be necessary to change the sampling ratio fairly often to meet the needs of field conditions. It is important, however, to record the exact procedure used each time so that changes in ratios can be taken into consideration when the time comes for analyses of the data. Further details are given in chapter 3.

VII. Special Biochemical Evaluations

A portion of the biochemical laboratory's capacity should be reserved for *special studies* of individuals whose clinical status indicates they may be of special interest in providing a basis for the interpretation of the findings from the routine procedures of the survey. Such individuals might be: (1) persons with frank deficiency disease; or (2) persons with certain clinical signs of "subclinical" deficiency, the interpretation of which would be aided by biochemical evidence on a series of similar cases. These individuals could be chosen from any of the subjects receiving clinical examination. If chosen from the individuals in the "abbreviated clinical examination" groups, a detailed clinical examination should be made. Records of such examination of "nonrandom" subjects would, of course, be kept separate from the records of individuals in the "random" sample.

Also, the resultant biochemical data, (the routine laboratory determinations are used for the random sample, or special biochemical determinations, load tests, or results of controlled therapeutic trials), should be kept separate from data obtained from the random sample.

Up to 25 percent of the biochemical facilities of the team may most profitably be engaged in such special-study analyses. Preliminary analyses of the biochemical data (such as ascorbic acid levels) during the survey may reveal clear evidence of the biochemical status of the Forces for the measurement. In such a case it may be permissible to do future determinations of this measurement on only every second or third blood sample taken thereafter. The time so saved may be used for special-study work.

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Clinical Appraisal of Nutriture

I. Necessity for Standardization of Diagnostic Criteria

In numerous surveys it has been demonstrated that one of the most important sources of confusion and differences in physical findings between groups, is the individual examiner's idiosyncrasy of definition and recognition of clinical signs. The recognition of these spurious differences between groups leads to confusion of interpretation, failure to recognize them leads to misinterpretation. Accordingly, it is essential to eliminate them, or at least to minimize them. Hence, the necessity for clear definition and standardization of observations cannot be overemphasized.

The definition of clinical signs to be recorded is a matter for joint decision by the nutritional examiners who will direct and perform the surveys. Once this decision is made, it must be adhered to by all groups throughout the period of the study. In order that these defined signs shall be comparably recognized, there must be planned standardization sessions at which each examiner independently examines and records his observations on a sample of some 100 persons. The identical group of examinees must be examined by all examiners. At the conclusion of the examinations the results of the several examiners are compared and joint re-examination made of those subjects upon whom inconsistencies are obtained, in order to allow formulation and agreement of specifications for recording.

Such a session should be held not only prior to initiation of the study but also at intervals throughout the survey in order to recognize any divergence from agreed definitions which may occur as the different examiners proceed with their work. A minimum of such standardization sessions would be: (1) at the initiation of the study; and (2) at the conclusion.

Experience demonstrates that there is less likelihood of divergence of opinion or of standards by examiners in the instances of frank deficiency diseases, but that those characteristics which depend upon slight difference in color, in texture, in dryness, in pigmentation, in vascularity, and the like, are susceptible to pronounced differences of opinion in recording.

Another source of error which frequently occurs is the tendency to interpret findings at the time of observation, and thereby to decide whether a particular sign should or should not be recorded.

It is well established that an individual examiner's criterion for recording a specific observation alters, depending upon the immediately recent background of experience of the individual.

Thus, as one encounters a group in which a particular sign is very frequent, he may be less impressed by the mild lesions after examining the group for a period, than he was at the beginning of his examinations. For example, physicians are inclined to alter their "standards of judgment of normal weight" rather rapidly if they are constantly studying a lean or an obese population. Many other examples of this problem

may be cited, but the following accounts give evidence of the importance of the problem in making surveys.

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II. Clinical Examination Procedures

The space and personnel requirements to be given in this section are designed to allow clinical examination of an adequate sampling of individuals. The total sample of persons chosen for examination at each location will be processed through a "main line," with 4 out of every 5 subjects receiving an *abbreviated examination.* Every fifth man in this "main line" will be shunted off to a second area, there to receive a *detailed examination.* It should be possible in the main line to accommodate a total of 500 to 600 individuals per working day, with 100 to 120 of them receiving detailed examinations.

Subjects selected for the *main sample* should be brought to the area selected for the location of the abbreviated examination facilities in groups of around 100. If entire company units are drawn for the sample, they will be the appropriate size of groups. To facilitate an even flow of individuals, it may be necessary to set up on the day preceding examinations, a schedule for arrival of units of men at the examination area. If the men are administratively divisible into units of 100 or so, then units might be scheduled for arrival at 8, 9:30, and 10:30 in the morning, and at 1:30 and 2:30 in the afternoon.

Four of every five individuals in this main sample will be given the abbreviated clinical examination. This will be limited to selected major signs of nutritional significance, the choice of signs for inspection being made for each country according to prior knowledge of which types of deficiency are more likely to be encountered. Age, height, and weight data will be recorded for each examinee.

Individual record cards may be prepared using a small card (either McBee edge-punched cards or simple file cards) with the brief examination form chosen as appropriate for the country, stenciled onto the card. (Alternately, bound notebooks may be adapted for an adequate record.) Location and activity of each group of subjects should be recorded.

Survey identification numbers will be assigned the subjects in a non-overlapping manner. The numbering system will begin with the McBee edge-punched cards for the recording of the detailed clinical examination, continue on the specially colored McBee edge-punched cards for individuals of special interest, described in the next paragraph, and end with the cards for the abbreviated examination. All cards will be numbered before the beginning of the survey.

Thus, if sample size to be attained was 15,000, with 10,000 abbreviated clinical examinations, 3,000 detailed clinical examinations and 2,000 special examinations, the regular McBee cards would be numbered consecutively from 0001 to 3,000, while the specially colored McBee cards would follow with numbers 3,001 to 5,000 and the cards for the abbreviated examination would be numbered consecutively, 5,001 to 15,000.

Four out of every five persons in the main sample will be given the small card. Every fifth individual will be given the large McBee edge-punched card and will be directed to proceed to the other examination area where the detailed clinical examinations will be done.

Individuals among the 80 percent receiving only the abbreviated examination, who, because of special interest indicated by the findings of the abbreviated inspection, are considered appropriate for special study, will be given one of the large McBee cards and sent to receive the detailed clinical examination, biochemical sampling, and/or whatever special therapeutic tests or other nutritional evaluation which may be selected. McBee cards printed in a different color than those used for the detailed examination will be used for these individuals, to make sure that their records are kept separate from those of individuals in the random sample.

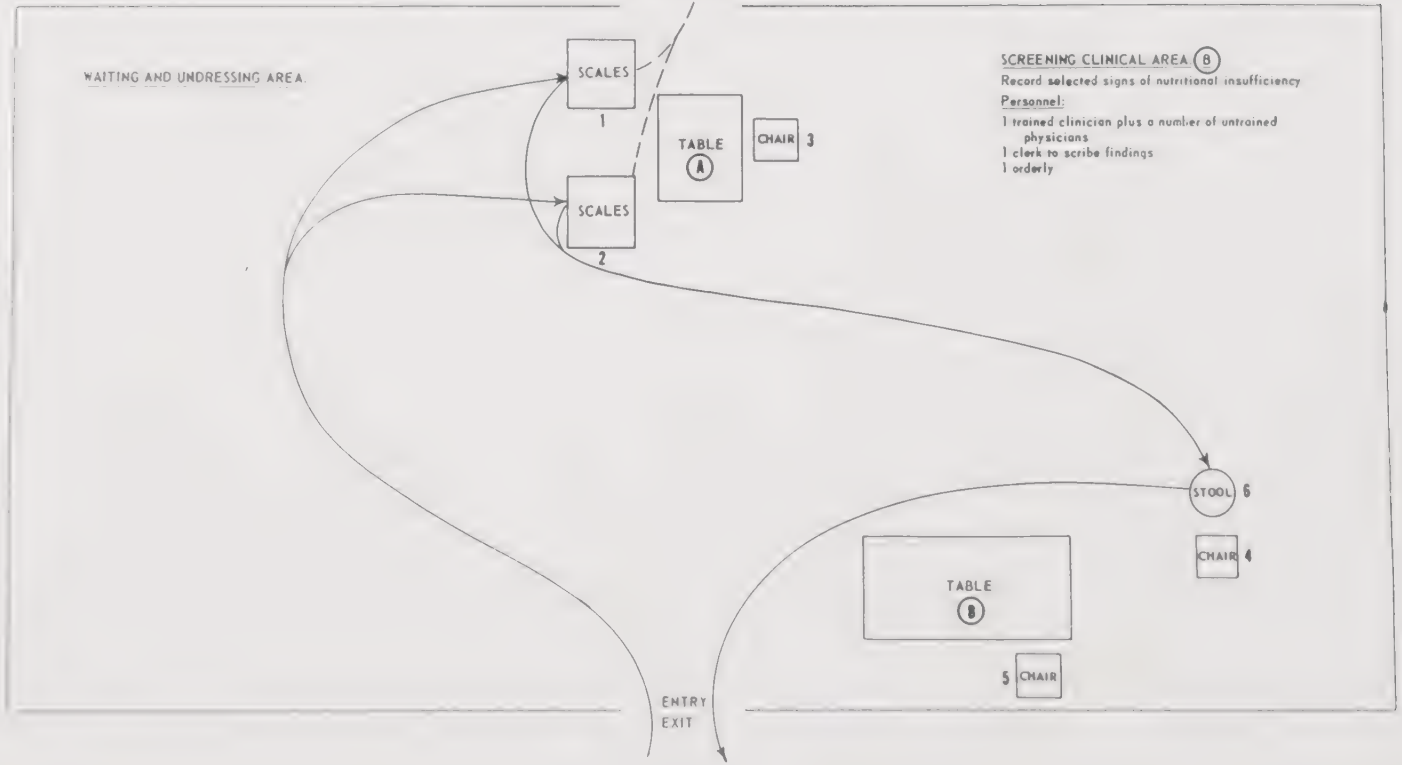
Requirements of space, equipment, and personnel for the two examination areas, with diagrams of suggested layouts follow on pages 16 and 18.

ABBREVIATED CLINICAL EXAMINATION AREA

SCREENING IDENTIFICATION AREA: (A)
Measure and record height, weight and age of each man
Record location and activity of each group

Personnel:
Secretary - interpreter
2 clerk technicians

Every fifth man to detailed clinical exam



1. Abbreviated Clinical Examinations

(See chart on "Abbreviated Clinical Examinations," p. 16)

a. Facilities

A well-lighted (both with sunlight and artificial light) structure is required, either permanent or under canvas, with at least 800 square feet of floor space and an adequate ceiling height.

b. Personnel Required—Minimum of 6 as follows:

(1) *Clinicians* (B4). One physician trained in nutritional examinations, plus one or more untrained physicians. If only one trained clinician is available for both the examination areas, a preliminary training period for the untrained physicians, of at least one week's duration, might permit the latter to sort out the interesting nutritional cases. Then a 10-minute-every-hour consultation with the trained clinician could confirm the findings.

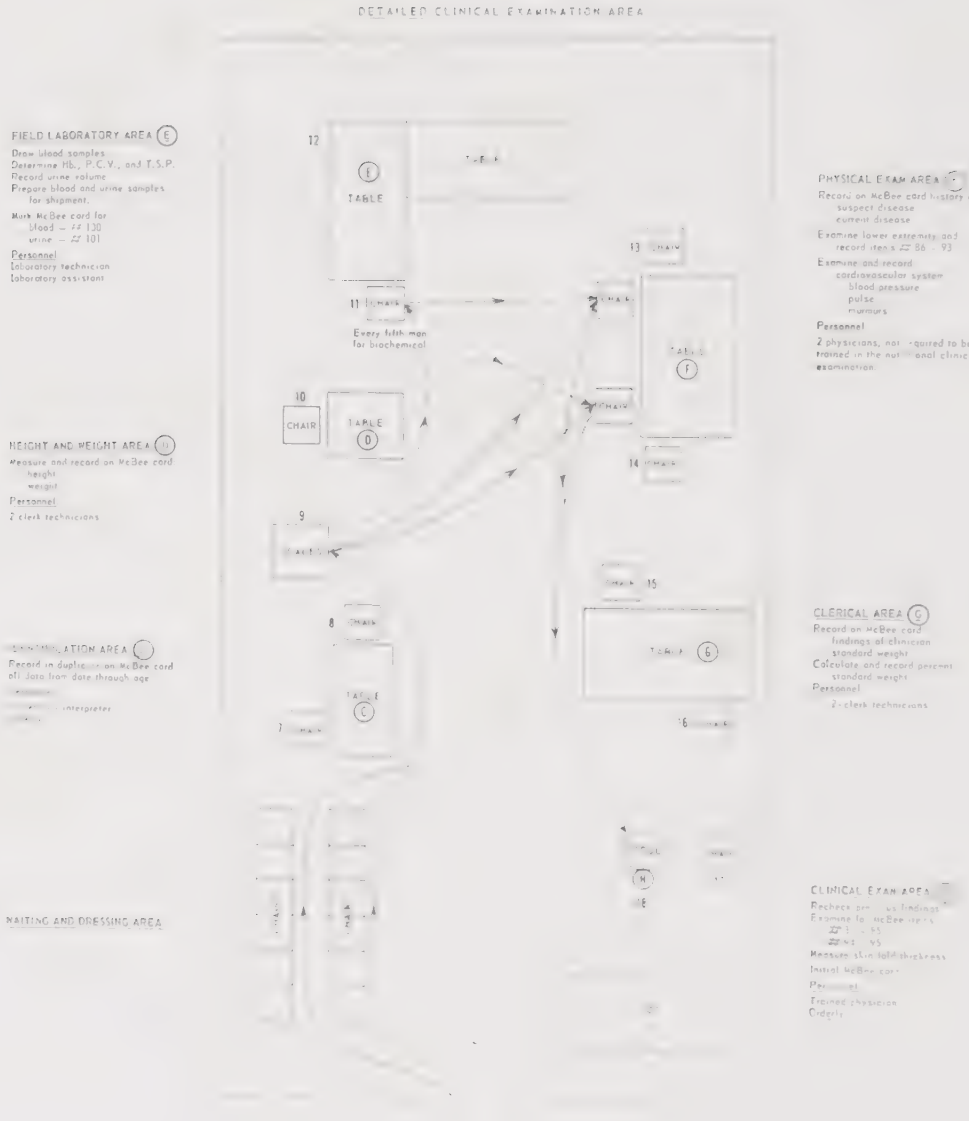
(2) *Secretary-Interpreter* (A3). One intelligent bilingual (both spoken and written) person to record age, height, and weight.

(3) *Clerk-Technicians* (A1), (A2). Two, to be trained to measure height and weight. (B5) One, to scribe for the clinician.

(4) *Orderly* (B6). One, to facilitate movement of the examinees, and to serve as a messenger between the examination areas.

c. Equipment

(1) 1 weight scale—in pounds
(2) 1 height tape measure
(3) 1 stethoscope
(4) 1 reflex hammer
(5) 1 vibratory tuning fork—128/min.
(6) 1 flashlight
(7) 500 tongue blades per working day
(8) 3 record books and 500 abbreviated examination cards
(9) 6 ballpoint pens and pencils
(10) 1 slide rule
(11) 6 extra copies of table 1.
(12) 1 table, 4 feet by 3 feet
(13) 1 table, 6 feet by 3 feet
(14) 2 steel wastepaper baskets
(15) 5 chairs
(16) 1 washbasin, soap and towel


DETAILED CLINICAL EXAMINATION AREA
FIELD LABORATORY AREA (E)
Draw blood samples
Determine Hb., P.C.V., and T.S.P.
Record urine volume
Prepare blood and urine samples for shipment.
Mark McBee card for
blood — # 130
urine — # 101
Personnel
Laboratory technician
Laboratory assistant
HEIGHT AND WEIGHT AREA (D)
Measure and record on McBee card
height
weight
Personnel
2 clerk technicians
INTERVIEW AREA (B)
Record in duplicate on McBee card all data from date through age
interpreter
WAITING AND DRESSING AREA
PHYSICAL EXAM AREA (F)
Record on McBee card history of
suspect disease
current disease
Examine lower extremity and record items # 86 - 93
Examine and record cardiovascular system
blood pressure
pulse
murmurs
Personnel
2 physicians, not required to be trained in the nutritional clinical examination
CLERICAL AREA (G)
Record on McBee card findings of clinician
standard weight
Calculate and record percent standard weight
Personnel
2-clerk technicians
CLINICAL EXAM AREA
Recheck previous findings
Examine for McBee items
1 - 85
94 - 95
Measure skin fold thickness
Initial McBee card
Personnel
Trained physician
Orderly
TABLE
CHAIR
Every fifth man for biochemical
12
13
11
10
9
8
7
14
15
6

2. Detailed Clinical Examination

(See chart on "Detailed Clinical Examinations," p. 18)

Every fifth subject in the "main line" sample will be sent to the detailed clinical examination area. All these will have identification data recorded (Area C), physical examination (Area F), and clinical examination (Area H). In addition, one man in every five (e.g., one-twenty-fifth of the main sample) will have blood samples taken for biochemical assessment (Area E) and be listed for subsequent urine sample collection (*see* Biochemical Methods chapter).

a. Facilities

A well-lighted (both with sunlight and artificial light) structure is required, either permanent or under canvas, with at least 800 square feet of floor space and an adequate ceiling height.

b. Personnel Required—12 persons as follows:

(1) *Clinician* (H17). One physician trained in nutritional examinations. This clinician's main responsibility is in the detailed clinical examination phase. If necessary he can supervise the clinical examination for selected signs of nutritional insufficiencies in the abbreviated phase.

(2) *Physicians* (F13, 14). Two, to examine and record suspected or current disease, the cardiovascular system and the lower extremities.

(3) *Field Laboratory Personnel* (E11). One technician; (E12) one assistant.

(4) *Secretary-Interpreter* (C7). One intelligent and bilingual (both spoken and written) person to record all identification data on McBee card from date through age.

(5) *Clerk-Technician* (D9, 10). Two, to measure and record height and weight; (G15) one, to scribe for clinician; (G16) one, to record standard weight and possibly to calculate percentages of standard weight. As time allows, this clerk should also record standard weight on the records of the subjects receiving only the abbreviated examination.

(6) *Orderlies* (C8). One, to facilitate movement of the examinees, to assist the secretary-interpreter, to prepare cards with carbons, to procure supplies, and to serve as messenger between the two examination areas. (H18) One, to assist clinician with examination.

c. Equipment and Supplies

(1) 1 weight scale—in pounds
(2) 1 height and tape measure
(3) 2 blood pressure sphygmomanometers
(4) 2 watches with second hand
(5) 2 stethoscopes
(6) 2 reflex hammers
(7) 2 tuning forks—128/min.

(8) 150 tongue blades per working day
(9) 3 flashlights and extra batteries
(10) 1 skinfold caliper and 70 g. wt. for standardization
(11) 1 otoscope with ocular attachments
(12) 2 cameras with flash accessories (a) Leica type with nooky attachment and 50 mm. and 35 mm. lenses; (b) a single lens reflex with extension tubes.
(13) 2 tables 4 feet by 3 feet
(14) 4 tables 6 feet by 3 feet
(15) 3 steel wastepaper baskets
(16) 1 cot and blanket
(17) 1 washbasin, soap and towel
(18) 20 chairs
(19) 1 record book—for serial and card number cross check
(20) 1 typewriter
(21) 300 McBee cards per working day
(22) McBee card repair kit
(23) 6 punches for McBee cards and sorting needles
(24) Carbon paper
(25) Pencils and pencil sharpener
(26) 9 ballpoint pens
(27) Paper—typewriter (8½″ x 11″); pads (scratch); pads (8½″ x 11″); envelopes; clips
(28) Erasers
(29) Stamps (postage)
(30) Cleansing tissue
(31) Twine
(32) Masking tape and cellulose tape
(33) 1 slide rule
(34) 12 extra copies of tables 1 and 2a–2k.
(35) 6 manila file folders
(36) 2 date stamps and ink pads
(37) 3 right angle triangles

III. Definitions and Instructions for Recording Data from Detailed and Abbreviated Physical Examinations

All data obtained in the detailed clinical examination of each individual are to be recorded in duplicate on McBee record cards. (See McBee card.) Additional instructions for the coding and punching of the cards for use in the analysis of the data will be given in section IV of this chapter. The card has space at the bottom of each side for an additional coding of the data, for detailed analysis using I. B. M. (International Business Machine) equipment; this coding will be explained in section V of this chapter.

The McBee cards are prepared in attached-pairs, with perforation along one edge. They are to be folded over and a carbon inserted for

making a duplicate copy. The duplicate file will be left in the country surveyed. The abbreviated physical examination card (unpunched) is not duplicated.

The identification data at the top of the front of the card can be obtained and recorded by a bilingual secretary, after instruction in definition of terms and methods for recording. It should be verified periodically that the secretary is adhering to the instructions. All entries through age should be recorded by this individual. (A date-stamp may be used for the date entry. A number-stamp may be used for entering numbers in the space for card number. The other identification data should be typed if possible.)

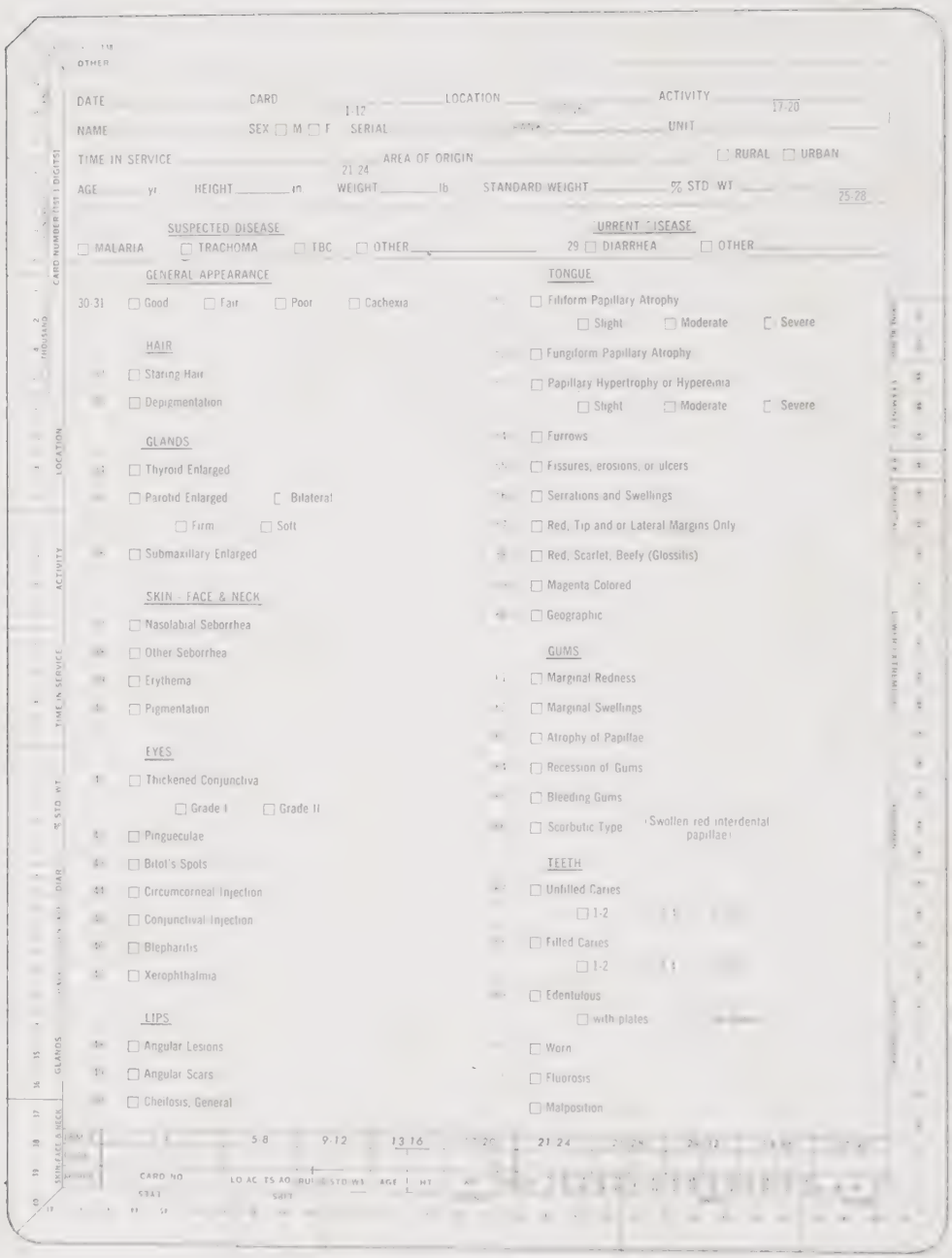
Height and weight can be measured and recorded by "Clerk-Technicians," with initial training in the methods of measurement and procedures for recording. Their work should be subjected to periodic checking to verify that instructions are being followed. Numerals should be written so as to be unambiguously legible.

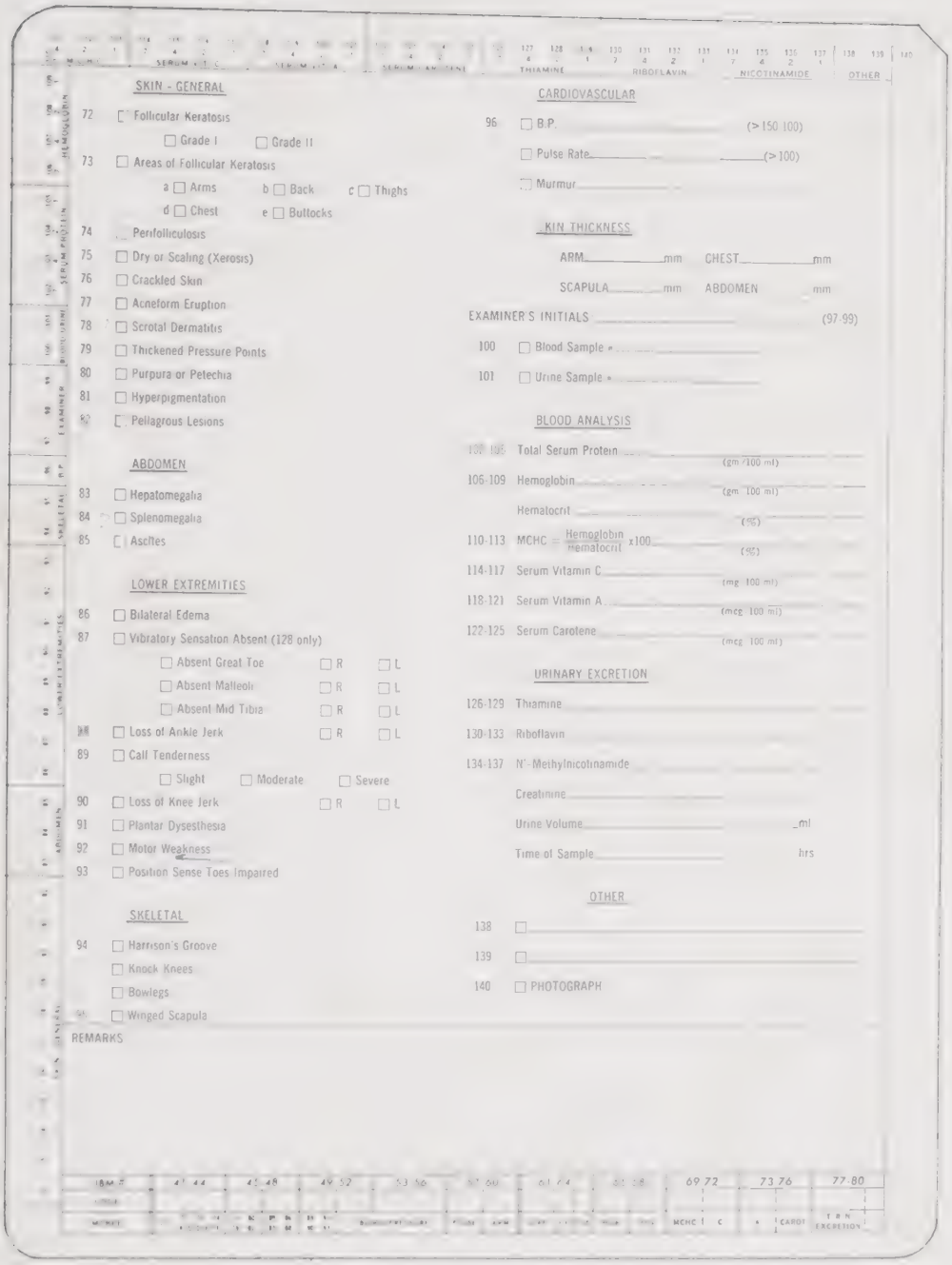
Standard weight, for given age and height, will be obtained from table 1, which gives the Medico-Actuarial "standards." Conversion tables for determining percentage of standard weight for ages 20–30 are given in tables 2a–2k. The personnel requirements as listed in the preceding section provide for a clerk to enter the standard weights on the cards, to calculate by slide rule each individual's percentage of standard weight, and to code the percentages for punching into the margin of the McBee card. Fractions of a percent are to be dropped in these calculations, to enable proper coding of the data into intervals of 10 percent. All these operations should be carefully checked for accuracy, by complete and independent repetition of the procedures. After completion of the survey, all computations will be rechecked using a calculating machine before the data are recorded on the I. B. M. code. If a calculating machine is available at any of the field locations, its use can replace the slide rule calculations, with repetition to check accuracy, and with immediate recording on the I. B. M. code. This need be done for only those individuals not included in tables 2a–2k.

Positive history or direct evidence of suspected malaria, trachoma, or tuberculosis, and current presence of diarrhea should be examined for by physicians. Space is provided on the McBee card for recording of other diseases seen, which, if thought relevant to the evaluation of nutritional status, should be recorded. Pertinent details concerning any positive findings in this section may be recorded in the space labelled "Remarks" on the back of the card. The physicians doing this portion of the examination can also perform the examinations of the cardiovascular system and the lower extremities.

During the physical examination a mark should be made for each sign or finding for which examination has been made. If a sign is *present*, an "X" should be placed in the square box immediately to the left of its listing on the card. If the sign is *absent*, (negative), a check ($\surd$) should be placed after its listing to indicate that the examination was made. On completion of the examination, the clinician should verify the completeness of the record before initialing the card.

Some of the items on the punch card are self-explanatory. Others require comment or special instruction if the recording is to be uniform.


OTHER
DATE
CARD
1-12
LOCATION
ACTIVITY
17-20
NAME
SEX ☐ M ☐ F
SERIAL
UNIT
TIME IN SERVICE
AREA OF ORIGIN
21-24
☐ RURAL ☐ URBAN
AGE yr
HEIGHT in
WEIGHT lb
STANDARD WEIGHT
% STD WT
25-28
SUSPECTED DISEASE
☐ MALARIA ☐ TRACHOMA ☐ TBC ☐ OTHER
CURRENT DISEASE
29 ☐ DIARRHEA ☐ OTHER
GENERAL APPEARANCE
30-31 ☐ Good ☐ Fair ☐ Poor ☐ Cachexia
HAIR
☐ Staring Hair
☐ Depigmentation
GLANDS
☐ Thyroid Enlarged
☐ Parotid Enlarged ☐ Bilateral
☐ Firm ☐ Soft
☐ Submaxillary Enlarged
SKIN - FACE & NECK
☐ Nasolabial Seborrhea
☐ Other Seborrhea
☐ Erythema
☐ Pigmentation
EYES
☐ Thickened Conjunctiva
☐ Grade I ☐ Grade II
☐ Pingueculae
☐ Bitot's Spots
☐ Circumcorneal Injection
☐ Conjunctival Injection
☐ Blepharitis
☐ Xerophthalmia
LIPS
☐ Angular Lesions
☐ Angular Scars
☐ Cheilosis, General
TONGUE
☐ Filiform Papillary Atrophy
☐ Slight ☐ Moderate ☐ Severe
☐ Fungiform Papillary Atrophy
☐ Papillary Hypertrophy or Hyperemia
☐ Slight ☐ Moderate ☐ Severe
☐ Furrows
☐ Fissures, erosions, or ulcers
☐ Serrations and Swellings
☐ Red, Tip and or Lateral Margins Only
☐ Red, Scarlet, Beefy (Glossitis)
☐ Magenta Colored
☐ Geographic
GUMS
☐ Marginal Redness
☐ Marginal Swellings
☐ Atrophy of Papillae
☐ Recession of Gums
☐ Bleeding Gums
☐ Scorbutic Type (Swollen red interdental papillae)
TEETH
☐ Unfilled Caries
☐ 1-2
☐ Filled Caries
☐ 1-2
☐ Edentulous
☐ with plates
☐ Worn
☐ Fluorosis
☐ Malposition
CARD NO
LO AC TS AO BU STD WT AGE HT
SEX



SKIN - GENERAL

72 ☐ Follicular Keratosis
☐ Grade I ☐ Grade II

73 ☐ Areas of Follicular Keratosis
a ☐ Arms b ☐ Back c ☐ Thighs
d ☐ Chest e ☐ Buttocks

74 ☐ Perifolliculosis
75 ☐ Dry or Scaling (Xerosis)
76 ☐ Crackled Skin
77 ☐ Acneform Eruption
78 ☐ Scrotal Dermatitis
79 ☐ Thickened Pressure Points
80 ☐ Purpura or Petechia
81 ☐ Hyperpigmentation
82 ☐ Pellagrous Lesions

ABDOMEN

83 ☐ Hepatomegalia
84 ☐ Splenomegalia
85 ☐ Ascites

LOWER EXTREMITIES

86 ☐ Bilateral Edema
87 ☐ Vibratory Sensation Absent (128 only)
☐ Absent Great Toe ☐ R ☐ L
☐ Absent Malleoli ☐ R ☐ L
☐ Absent Mid Tibia ☐ R ☐ L
88 ☐ Loss of Ankle Jerk ☐ R ☐ L
89 ☐ Calf Tenderness
☐ Slight ☐ Moderate ☐ Severe
90 ☐ Loss of Knee Jerk ☐ R ☐ L
91 ☐ Plantar Dysesthesia
92 ☐ Motor Weakness
93 ☐ Position Sense Toes Impaired

SKELETAL

94 ☐ Harrison's Groove
☐ Knock Knees
☐ Bowlegs
95 ☐ Winged Scapula

REMARKS

CARDIOVASCULAR

96 ☐ B.P. _____ (> 150 100)
☐ Pulse Rate _____ (> 100)
☐ Murmur _____

SKIN THICKNESS

ARM _____ mm CHEST _____ mm
SCAPULA _____ mm ABDOMEN _____ mm

EXAMINER'S INITIALS _____ (97-99)

100 ☐ Blood Sample # _____
101 ☐ Urine Sample # _____

BLOOD ANALYSIS

102-105 Total Serum Protein _____ (gm /100 ml)
106-109 Hemoglobin _____ (gm 100 ml)
Hematocrit _____ (%)
110-113 MCHC = $\frac{\text{Hemoglobin}}{\text{Hematocrit}}$ x100 _____ (%)
114-117 Serum Vitamin C _____ (mg 100 ml)
118-121 Serum Vitamin A _____ (mcg 100 ml)
122-125 Serum Carotene _____ (mcg 100 ml)

URINARY EXCRETION

126-129 Thiamine _____
130-133 Riboflavin _____
134-137 N'-Methylnicotinamide _____
Creatinine _____
Urine Volume _____ ml
Time of Sample _____ hrs

OTHER

138 ☐ _____
139 ☐ _____
140 ☐ PHOTOGRAPH

ABBREVIATED PHYSICAL EXAMINATION CARD

DATE _____ CARD # _____ (1-5) LOCATION _____ (6) ACTIVITY _____ (7)

NAME _____ SEX ☐ M ☐ F RANK _____ UNIT _____

TIME IN SERVICE _____ (8) AREA OF ORIGIN _____ (9) ☐ RURAL (10) ☐ URBAN

AGE _____ (11-12) YR. HT. _____ (13-14) IN. WT. _____ (15-17) LBS. STD. WT. _____ (18-20) %STD. WT. _____ (21-23)

24 GLANDS
☐ Thyroid Enlarged

25 SKIN - FACE & NECK
☐ Nasolabial Seborrhea

26 EYES
☐ Bitot's Spots

27 LIPS
☐ Angular Lesions
☐ Angular Scars
☐ Cheilosis, General

28 TONGUE
☐ Magenta Colored
☐ Filiform Papillary Atrophy
☐ Slight ☐ Moderate ☐ Severe

29 ☐ Glossitis

30 GUMS
☐ Scorbutic Type

31 SKIN - GENERAL
☐ Follicular Keratosis
☐ Arms ☐ Back

32
☐ Scrotal Dermatitis
☐ Pellagrous Lesions

33 LOWER EXTREMITIES
☐ Bilateral Edema
☐ Loss of Ankle Jerk ☐ R ☐ L

34 ☐ Calf Tenderness
☐ Slight ☐ Moderate ☐ Severe

35 ☐ Studied Further

Special Card No. _____ (36-40)

ITEMS	1 - 5	6 - 10	11 - 17	18 - 23	24 - 29	30 - 34	35 - 40
CODE							
ITEM	CARD #	LO AC TS AO RU	AGE HT. WT.	STD.WT. %STD.WT.	T NS B LIPS ME G TONGUE	F SP BE-A CT GUMS SKIN L. EXT.	SF CARD NO

ICNND-7

McBee	*I.B.M.*	
		Date.—Record month, day, and year, in that order.
1–12	1–4	*Card No.*—Record consecutive numbers to identify card and individual clearly. Use four digit numbers; e.g., 0001, 0013, 0135, 1357, on the McBee card and five digit numbers on the abbreviated clinical card.
13–16	5	*Location.*—Set up code for this item and enter code number directly on the card. This item refers to place or area where examination was performed.
17–20	6	*Activity.*—Set up code for this item and enter code number directly on the card. This item permits grouping by type of physical activity or environmental factors or organizational units within the Armed Forces, which may influence nutritional requirements or nutritional status.
..	.	*Name, sex, serial number, unit.*—These items are to be recorded on the card, but not punched. They are designed to identify the individual by family name, serial number, and unit (such as division, regiment, battalion, company, etc.).
21–24	7	*Time in service.*—Enter on card in years and months. Code according to plan provided in section IV and enter code number on card.
..	8	*Area of origin.*—Set up code for this item and enter code number directly on card. This item is to be used to group individuals according to origin at time of induction. This may be geographic, by Province, or by ethnic origin, whichever has greater significance in terms of food practices, food availability, or other characteristics which might influence nutritional status at the time of induction.
.	9	*Rural-urban.*—Rural refers to dwelling in the country where home-produced foods constitute the major portion of the diet. Urban refers to dwelling in a village, town or city where foods are purchased at a market and where there was little dependence on home-produced foods at the soldier's area of origin.
..	13–14	*Age.*—If known, enter the age at last birthday. If unknown, estimate at less than 21 or greater than 21 years. Note must be taken that age is calculated in different ways in some countries. Record all values in equivalent American age.
..	15–16	*Height.*—Record in inches to the *nearest whole inch;* measure without shoes, using a right angle triangle. In cases where the measurement is *exactly* on a half inch, record to the nearest *even inch,* e.g., 63½ inches is recorded as 64 inches; 62½ inches as 62 inches.
..	17–19	*Weight.*—Record in pounds to the *nearest whole pound,* the weight being taken without clothing, except for shorts. In cases where the weight is *exactly* on a half pound, record to the *nearest even pound,* e.g., 138½ pounds is recorded as 138; 139½ pounds as 140 pounds.
..	..	*Standard weight.*—This can be entered as soon as convenient after the examination, and will be the appropriate number from table 1. This table has been calculated from the Medico-Actuarial tables by subtracting 1 inch and 5 pounds from each height, weight, age category, to allow for height being measured without shoes, and the weight being measured without clothing.
25–28	10–12	*Percentage of standard weight.*—This item should be completed as the data are being checked and the McBee cards punched for analysis. Compute the percentage weight by slide rule, dropping all fractions and record to the next lowest whole percent, e.g., 97.3 is recorded as 97; 108.9 is recorded as 108. Percent standard weight may be read directly from tables 2a–2k for the majority of troops. Code for the McBee card as provided in section IV and enter on the card. Leave blank on the abbreviated form as this will be calculated by I.B.M. later.

..	20	*Suspected disease.*—Record if there is a positive history or definite evidence of the presence of the diseases listed on card or other diseases that may be significant.
29	21	*Current disease.*—Record if there is a positive history or definite evidence of the presence of diarrhea at the time of the examination.
30–31	22	*General appearance.*—Base classification on overall mental and physical appearance in the light of what one would expect to see in healthy United States' males of similar age.

Note on clinical signs.—Each team will have a set of colored lantern slides which will illustrate most, if not all, of the lesions. The following notes will concern special instructions regarding each item. Further information may be obtained from standard references.

HAIR

32	23	*Staring hair.*—Lusterless, dry, unruly, brittle hair.
33	24	*Depigmentation of the hair.*—Do not record in military populations. Depigmentation of the hair in infants may result in distinctive white bands, or in the lightening of normally black hair to a brownish or reddish color. The term "achromotrichia" has been applied to the whitening or graying of the hair. There may be associated texture changes, especially in the Negro, in whom the coarse, firm, kinky, wooly hair becomes thinner, finer in texture and lighter in color.

GLANDS

34	24	*Thyroid enlarged.*—Must be definitely palpable with or without swallowing and at least slightly perceptible to visual inspection.
35	24	*Parotid enlarged.*—Because of various types of facial configuration, parotid enlargement may be easily missed in certain populations. Check by palpation, moving the gland with fingers upwards and backwards toward the ear.
36	24	*Submaxillary enlarged.*—Detect by both inspection and palpation.

SKIN—FACE AND NECK

37	25	*Nasolabial seborrhea.*—Definite greasy yellowish scaling or filiform excresences in the nasolabial area which becomes more pronounced on slight scratching with the fingernail or a tongue blade.
38	25	*Other seborrhea.*—Look especially behind ears, at outer canthus of eyes, forehead, in axillae and intergluteal region. Do not confuse with fungus infections. Seborrhea of the scalp is not to be recorded in military groups.
39	26	*Erythema.*—Splotchy erythematous areas, especially over malar eminences. Do not record simple sunburn or chapping.
40	26	*Pigmentation.*—Areas of light brown, mottled, patchy pigmentation about eyes—frequently over the malar eminences.

EYES

41	27	*Thickened conjunctivae.*—All types and degrees of thickening may occur. The blueness of the sclera may disappear, the bulbar conjunctiva develops a wrinkled appearance with what seems to be an increase of vascularity. The thickened conjunctiva may result in a glazed porcelainlike appearance, obscuring the vascularity. Do not confuse with pterygium.
42	28	*Pingueculae.*—Small circumscribed slightly raised spots, yellow or pigmented in color, in the exposed area of a moist conjunctiva.
43	28	*Bitot's spots.*—Small (1–3 mm.) circumscribed grayish or yellowish-gray, dull, dry, foamy lesions of the conjunctiva. Seen most often in the lateral aspect of the bulbar conjunctiva.
44	28	*Circumcorneal injection.*—Bilateral increase in vascularity with particular concentration around the cornea in the absence of trachoma, obvious infection, etc.

45 29 *Conjunctival injection.*—Generalized increase in the vascularity of the globe of the eye—in the absence of obvious infection, trachoma, etc.

46 29 *Blepharitis.*

47 29 *Xerophthalmia.*—Dryness of the conjunctiva, with lack of tearing, frequently associated with infection and sometimes with photophobia.

LIPS

48 30 *Angular lesions.*—Record only if definitely present bilaterally when mouth is held one-half open. Beware of confusion which may arise in examining the denture wearing individual.

49 30 *Angular scars.*—Scars at the angles, which if recent, may be pink; if old, may appear blanched.

50 30 *Cheilosis.*—Different than ordinary chapping. The lips are swollen, tense, or puffy with desquamation, and look as if the buccal mucosa extends out onto the lip. May use this category to record inflammatory appearing vertical fissuring of the lips.

TONGUE

51 31 *Filiform papillary atrophy.*—The filiform papillae have disappeared or are exceedingly low, giving the tongue a smooth appearance which remains after scraping with an applicator stick. "Slight" involves less than one-fourth of the tongue, (tip and lateral margins only); "Moderate" involves one-fourth to three-fourths of the tongue; "Severe," over three-fourths of the tongue involved.

52 31 *Fungiform papillary atrophy.*—This is positive if the fungiform papillae cannot be readily seen. Attention must be given, however, to the variation in prominence of the fungiform papillae due to differences in height of filiform papillae.

53 23 *Papillary hypertrophy or hyperemia.*—Can be seen and felt with a tongue blade being drawn lightly over the anterior two-thirds of the tongue. Hyperemia refers to definite red dots.

54 32 *Furrows.*—Linear depressions without break in continuity of the epithelium. Papillae may be seen on the inside.

55 33 *Fissures.*—Cracks, with no papillae on sides or bottoms, and with a break in continuity of epithelium.

56 33 *Serrations.*—Tooth impressions at sides or tip often noted when tongue is swollen.

57 33 *Red, tip and/or lateral margins only.*

58 34 *Red, scarlet, beefy (glossitis).*—Entire tongue is red. Not just a modification of the natural color due to the loss of papillae alone.

59 34 *Magenta colored.*—The color of alkaline phenolphthalein just before the end-point.

60 34 *Geographic tongue.*—Irregularly shaped and distributed areas of atrophy, with irregular white patches resembling leukoplakia.

GUMS

61 35 *Marginal redness.*—Definite red border along the edge of the gum.

62 35 *Marginal swellings.*—In early stages—limited to margin and/or interdental papillae. May be spongy or firm. Initially occurs anteriorly and anterio-laterally, in advanced stage—involves entire gum.

63 35 *Atrophy of interdental papillae.*—Record when not due to absence or abnormal spacing of teeth.

64 36 *Recession with (or without) debris.*—Record when fairly generalized.

65 36 *Bleeding gums.*—Abnormal gums with spontaneous bleeding or bleeding upon slight pressure with a swab stick.

66 36 *Scorbutic type.*—Red, spongy, swollen interdental papillae.

TEETH

67 37 *Unfilled caries.*—Use grading on punch card.

68 38 *Filled caries.*—Use grading on punch card.

69 39 *Edentulous.*—Absence of all natural teeth from one or both jaws.

70 40 *Worn.*—Abnormal flattening of biting surface of teeth.

71 40 *Fluorosis.*—Mottled white or brown areas with horizontal ridging.

.. 40 *Malposition.*—Sufficient to interfere with the bite.

SKIN—GENERAL

72 41 *Follicular keratosis.*—This lesion has been likened to "goose flesh" which is seen on chilling, but it is not generalized and does not disappear with brisk rubbing of the skin. Follicular keratosis is more readily detected by the sense of touch than by the eye. The skin is rough, with papillae formed by keratotic plugs which project from the hair follicles. The surrounding skin is dry and lacks the usual amount of moisture or oiliness. Differentiation from adolescent folliculosis can usually be made through recognition of the normal skin between the follicles in the adolescent disorder. It is distinguished from perifolliculosis of ascorbic acid deficiency by the ring of capillary congestion which occurs about each follicle in scorbutic perifolliculosis.

73 42–43 *Areas of follicular keratosis.*—Do not mark box in margin unless multiple areas are involved.

74 44 *Perifolliculosis.*—Congestion around the follicles which do not blanch upon pressure. (See above.) Early there is a ring of capillary engorgement which does not disappear on pressure, around some hair follicles. It is, therefore, limited to the hairy regions of the body, more frequently to the dependent parts such as the legs. Later, swelling and hypertrophy of the follicles occur, at which time the skin becomes rough. Follicular keratosis and perifolliculosis may co-exist.

75 44 *Xerosis.*—Xerosis is a clinical term used to describe a dry and crinkled skin which is accentuated by pushing the skin parallel to its surface. In more advanced cases, the often mottled pigmented scaly or alligator-like pseudo plaques, usually not greater than 0.5 cm. in diameter become evident. Their nutritional significance is quite controversial. Differential diagnosis must be made from changes due to dirt and exposure and ichthyosis.

76 44 *Crackled skin ichthyosis.*—The scales here are larger in size than in xerosis. It is often congenital and is most prominent in cool weather. It is nonnutritional.

77 45 *Acneform eruption.*—The presence of acneform lesions in an active stage should be recorded. Inactive scars should not be recorded as an acneform eruption.

78 45 *Scrotal dermatitis.*—The scrotum usually must be rotated to see the lesions. Differentiate from fungus infections which usually extend onto the skin adjacent to the scrotum.

79 45 *Symmetrical, thickened, pigmented pressure points.*—Look especially at belt area, ischial tuberosities, sacrum and over greater trochanters. Do not record when found only on elbows and knees.

80 46 *Purpura or petechia.*

81 46 *Hyperpigmentation and acrocyanosis.*—Is asymptomatic with no inflammatory component. It is seen most frequently on the dorsum of the hands and lower forearms, particularly where there is poor skin hygiene. The skin is rough, dry, and often with a grayish cyanotic base. There is not the sharp line of demarcation at the border of the lesion such as one sees in pellagra. This condition is not related to pellagra.

82	46	*Pellagrous lesions.*—Record when symmetrical lesions typical of acute or chronic, mild or severe pellagra are observed.

ABDOMEN

(Examination should be made upon supine subject)

83	47	*Hepatomegalia.*—Record if liver edge is more than 2 cm. below the costal margin.
84	47	*Splenomegalia.*—Record if spleen is palpable.
85	47	*Ascites.*

LOWER EXTREMITIES [1]

86	48	*Bilateral edema.*—Record only if bilateral.
87	48	*Vibratory sensation absent.*—(128 vibrations per minute only).
88	49	*Loss of ankle jerk.*—Record only if absent after reinforcement.
89	49	*Calf tenderness.*—Record when definite bilateral evidence of painful sensation occurs upon squeezing firmly the calf muscles between the thumb and finger. Beware of effects of recent severe exercise, e.g., marching.
90	49	*Loss of knee jerk.*—Record only if absent after reinforcement.
91	50	*Plantar dysesthesia.*—A painful sensation resulting from a pin run lightly along the sole of the feet.
92	50	*Motor weakness.*—Subject's inability to squat and rise three or four times.
93	50	*Position sense of toes impaired.*—Record if bilateral.

SKELETAL

94	..	*Harrison's groove.*—Do not record in military age groups.
..	..	*Knock knees.*—Do not record in military age groups.
..	..	*Bowlegs.*—Do not record in military age groups.
95	..	*Winged scapula.*—As a result of loss of weight or muscle tone.

CARDIOVASCULAR

96	51–56	*Blood pressure.*—Take on right arm, with individual in sitting position. Record data to nearest even unit. Do not punch McBee card unless values exceed 150/100.
.	57–58	*Pulse rate.*—Count for 30 seconds, multiply by 2 and record.

SKINFOLD THICKNESS

Four sites are listed but only two or perhaps three sites will be chosen. The posterior arm and scapula sites are preferred. The site to be used will be decided in the field by the director of the team. Be sure that the calipers are calibrated so that the spring tension gives a pressure of 10 gms. per sq. mm. of jaw surface. The skinfolds are grasped between the thumb and index finger. The span of the grasp is dependent on the thickness of the skinfold. The size of the fold should be enough to include two thicknesses of skin and subcutaneous fat but not muscle or fascia. To insure against including such structures, when in doubt, have subject perform an act which would contract the underlying muscles. The application of the calipers is about 1 cm. from the fingers and at a depth approximately equal to the thickness of the fold. All folds are taken in the vertical plane except where the lines of Linn result in torsion of the skinfold, then the skinfold is taken along these lines. Record measurement in mm.

[1] Items 86-89 to be examined routinely. If any abnormality is noted, complete examination through item 93.

McBee	*I.B.M.*	
..	59–60	*Arm.*—Taken at the midposterior midpoint between the tip of the acromion and the tip of the olecranon with the elbow in 90° flexion.
..	61–62	*Scapula.*—This is taken at the tip of the scapula with the subject in a relaxed standing position. Here the lines of Linn usually require the fold to be taken in the diagonal plane.
..	..	*Chest.* (1) At the level of the xiphoid in the midaxillary line. (2) Midpoint between anterior crease of the axilla and the nipple. (3) Just adjacent to the nipple along the line of No. 2, but not including any glandular tissue.
..	..	*Abdomen.*—Waist level— (1) At the midaxillary line. (2) On the extension of the midclavicular line.
97–99	63	*Examiner.*—The clinician should initial the McBee card upon completion of each examination. In addition, there should be set up a code for examiners and the examiner's number entered on the card.
100–137	64–79	*Biochemical data.*—The recording and coding of the biochemical data will be discussed in section IV.
138–139	..	*Other.*—Use this space to record lesions of possible interest or significance not provided for otherwise on the form.
140	..	*Photograph.*—Check if a photograph is taken.
..	..	*Remarks.*—Record here any information or questions which may be important in further analysis and study. *Studied further.*—Check on the abbreviated clinical card if subject was chosen for a special detailed examination. *Special card No.*—For use on the abbreviated clinical card only. If subject was chosen for a special detailed examination, record number of McBee card used for the special examination. If no further examination was made, leave this portion blank.

TABLE 1.—*"Standard Weight" of men of different statures and ages, medico-actuarial tables, adjusted to nude weight, no shoes* [1]

[In pounds]

Height Inches	15 yr.	16 yr.	17 yr.	18 yr.	19 yr.	20 yr.	21 yr.	22 yr.	23 yr.	24 yr.	25 yr.	26 yr.	27 yr.	28 yr.	29 yr.	30 yr.	31 yr.	32 yr.	33 yr.	34 yr.
59	102	104	106	108	110	112	113	114	115	116	117	118	119	120	121	121	122	122	122	123
60	104	106	108	110	112	114	115	116	117	118	119	120	121	122	123	123	124	124	124	125
61	107	109	111	113	115	117	118	119	120	121	121	122	123	124	125	125	126	126	126	127
62	110	112	114	116	118	120	121	122	123	124	124	125	126	127	128	128	129	129	129	120
63	113	115	117	119	121	123	125	126	127	128	128	129	129	130	131	131	132	132	132	133
64	117	119	121	123	125	127	129	130	131	132	132	133	133	134	135	135	136	136	136	137
65	121	123	125	127	129	131	133	134	135	136	136	137	137	138	139	139	140	140	140	141
66	125	127	129	131	133	135	136	137	138	139	140	141	141	142	143	143	144	144	144	145
67	129	131	133	135	137	139	140	141	142	143	144	145	145	146	147	147	148	149	149	150
68	133	135	137	139	141	143	144	145	146	147	148	149	149	150	151	151	152	153	154	155
69	137	139	141	143	145	147	148	149	150	151	152	153	153	154	155	156	157	158	159	160
70	142	144	146	148	150	151	152	153	154	155	157	158	158	159	160	161	162	163	164	165
71	147	149	151	153	155	156	157	158	159	160	162	163	164	165	166	167	168	169	170	171
72	152	154	156	158	160	161	162	163	164	166	168	169	170	171	172	173	174	175	176	177
73	157	159	161	163	165	166	167	168	170	172	174	175	176	177	178	179	180	181	182	183
74	162	164	166	168	170	171	172	173	175	177	179	181	182	183	184	185	186	187	188	189
75	167	169	171	173	175	176	177	178	180	182	184	186	187	188	189	191	192	193	194	195
76	172	174	176	178	180	181	182	183	185	187	189	191	192	193	194	196	197	198	199	201

Height Inches	35 yr.	36 yr.	37 yr.	38 yr.	39 yr.	40 yr.	41 yr.	42 yr.	43 yr.	44 yr.	45 yr.	46 yr.	47 yr.	48 yr.	49 yr.	50 yr.	51 yr.	52 yr.	53 yr.	54+ yr.
59	123	124	124	125	125	126	126	127	127	128	128	129	129	129	129	129	130	130	130	130
60	125	126	126	127	127	128	128	129	129	130	130	131	131	131	131	131	132	132	132	132
61	127	128	128	129	129	130	130	131	131	132	132	133	133	133	133	133	134	134	134	134
62	130	131	131	132	132	133	133	134	134	135	135	136	136	136	136	136	137	137	137	137
63	133	134	135	135	135	135	136	137	137	138	138	139	139	139	139	139	140	140	140	140
64	137	138	139	139	139	140	140	141	141	142	142	143	143	143	143	143	144	144	144	144
65	141	142	143	143	143	144	144	145	145	146	146	147	147	147	147	147	148	148	148	148
66	145	146	147	147	147	148	148	149	149	150	150	151	151	151	151	151	152	152	152	153
67	150	151	152	152	152	153	153	154	154	155	155	156	156	156	156	156	157	157	157	158
68	155	156	157	157	157	158	158	159	159	160	160	161	161	161	161	161	162	162	162	163
69	160	161	162	162	162	163	163	164	164	165	165	166	166	166	166	166	167	167	167	168
70	165	166	167	168	168	169	169	170	170	171	171	172	172	172	172	172	173	173	173	173
71	171	172	173	174	174	175	175	176	176	177	177	178	178	178	178	178	179	179	179	179
72	177	178	179	180	180	181	181	182	182	183	183	184	185	185	185	185	186	186	186	186
73	184	185	186	187	187	188	188	189	189	190	190	191	192	192	192	192	193	193	193	193
74	190	191	192	193	194	195	195	196	196	197	197	198	199	199	199	199	200	200	200	200
75	196	197	198	199	200	201	202	203	203	204	204	205	206	206	206	206	207	207	207	207
76	202	203	204	205	206	207	208	209	209	210	210	211	212	212	212	212	213	213	213	214

[1] Association of Life Insurance Medical Directors and Actuarial Society of America: *Medico-Actuarial Mortality Investigation*, Vol. I, p. 38, New York, 1912. Tables are adjusted to approximate nude weight and no shoes by subtracting 5 pounds and 1 inch from each height-weight-age category.

TABLE 2a.—*Conversion table for percentage of standard weight*[1]*: 20-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	80	82	84	87	89	92	95	98	101	103	106	110	113
71	81	84	86	88	91	94	96	99	102	105	108	111	115
72	83	85	87	89	92	95	98	101	103	106	109	113	116
73	84	86	88	90	93	96	99	102	105	108	111	114	118
74	85	87	89	92	94	97	100	103	106	109	112	116	120
75	86	88	90	93	96	99	102	105	108	111	114	117	121
76	87	89	92	94	97	100	103	106	109	112	115	119	123
77	88	91	93	95	98	101	104	108	111	114	117	121	124
78	89	92	94	96	100	103	106	109	112	115	118	122	126
79	91	93	95	98	101	104	107	110	113	117	120	124	128
80	92	94	96	99	102	105	108	112	115	118	121	125	129
81	93	95	98	100	103	107	110	113	116	120	123	127	131
82	94	96	99	101	105	108	111	114	118	121	124	128	133
83	95	98	100	103	106	109	113	116	119	123	126	130	134
84	96	99	101	104	107	111	114	117	121	124	127	132	136
85	97	100	102	105	108	112	115	119	122	125	129	133	137
86	99	101	104	106	110	113	117	120	123	127	130	135	139
87	100	102	105	108	111	114	118	121	125	128	132	136	141
88	101	103	106	109	112	116	119	123	126	130	133	138	142
89	102	105	107	110	114	117	121	124	128	131	135	139	144
90	103	106	108	111	115	118	122	126	129	133	136	141	145
91	104	107	110	112	116	120	123	127	131	134	138	142	147
92	105	108	111	114	117	121	125	128	132	136	139	144	149
93	107	109	112	115	119	122	126	130	133	137	141	146	150
94	108	110	113	116	120	124	127	131	135	139	142	147	152
95	109	112	114	117	121	125	129	133	136	140	144	149	153
96	110	113	116	119	122	126	130	134	138	142	145	150	155
97	111	114	117	120	124	128	131	135	139	143	147	152	156
98	112	115	118	121	125	129	133	137	141	145	148	153	158
99	113	116	119	122	126	130	134	138	142	146	150	155	160
100	114	117	120	123	127	131	135	139	143	147	151	156	161
101	116	119	122	125	129	133	137	141	145	149	153	158	163
102	117	120	123	126	130	134	138	142	146	150	155	160	165
103	118	121	124	127	131	135	140	144	148	152	156	161	166
104	119	122	125	128	133	137	141	145	149	153	158	163	168
105	120	123	126	130	134	138	142	146	151	155	159	164	170
106	121	125	128	131	135	139	144	148	152	156	161	166	171
107	122	126	129	132	136	141	145	149	154	158	162	167	173
108	124	127	130	133	138	142	146	151	155	159	164	169	174
109	125	128	131	135	139	143	148	152	156	161	165	171	176
110	126	129	132–3	136	140	145	149	153–4	158	162–3	167	172–3	178

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2b.—*Conversion table for percentage of standard weight*[1]*: 21-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	81	83	85	88	91	94	96	98	101	104	107	110	114
71	82	84	86	89	92	95	97	100	103	106	108	112	116
72	83	85	88	90	93	96	98	101	104	107	110	114	117
73	84	87	89	92	95	98	100	103	106	109	111	115	119
74	86	88	90	93	96	99	101	104	107	110	113	117	120
75	87	89	91	94	97	100	102	105	108	111	114	118	122
76	88	90	92	95	99	102	104	107	110	113	116	120	124
77	89	91	94	97	100	103	105	108	111	114	118	121	125
78	90	93	95	98	101	104	107	110	113	116	119	123	127
79	91	94	96	99	102	106	108	111	114	117	121	125	128
80	92	95	97	100	104	107	109	112	116	119	122	126	130
81	94	96	99	102	105	108	111	114	117	120	124	128	132
82	95	97	100	103	106	110	112	115	119	122	125	129	133
83	96	98	101	104	108	111	113	117	120	123	127	131	135
84	97	100	102	105	109	112	115	118	121	125	128	132	137
85	98	101	103	107	110	114	116	119	123	126	130	134	138
86	99	102	105	108	111	115	117	121	124	128	131	136	140
87	101	103	106	109	113	116	119	122	126	129	133	137	141
88	102	104	107	110	114	118	120	124	127	131	134	139	143
89	103	106	108	112	115	119	122	125	129	132	136	140	145
90	104	107	109	113	117	120	123	126	130	134	137	142	146
91	105	108	111	114	118	122	124	128	132	135	139	143	148
92	106	109	112	115	119	123	126	129	133	137	140	145	150
93	107	110	113	117	120	124	127	131	134	138	142	147	151
94	109	111	114	118	122	126	128	132	136	140	143	148	153
95	110	113	115	119	123	127	130	133	137	141	145	150	154
96	111	114	117	120	124	128	131	135	139	143	146	151	156
97	112	115	118	122	126	130	132	136	140	144	148	153	158
98	113	116	119	123	127	131	134	138	142	146	149	154	159
99	114	117	120	124	128	132	135	139	143	147	151	156	161
100	115	118	121	125	129	133	136	140	144	148	152	157	162
101	117	120	123	127	131	135	138	142	146	150	154	159	164
102	118	121	124	128	132	136	139	143	147	151	156	161	166
103	119	122	125	129	133	137	141	145	149	153	157	162	167
104	120	123	126	130	135	139	142	146	150	154	159	164	169
105	121	124	128	132	136	140	143	147	152	156	160	165	171
106	122	126	129	133	137	141	145	149	153	157	162	167	172
107	124	127	130	134	139	143	146	150	155	159	163	168	174
108	125	128	131	135	140	144	147	152	156	160	165	170	175
109	126	129	132	137	141	145	149	153	157	162	166	172	177
110	127	130	134	138	142–3	147	150	154–5	159	163–4	168	173–4	179

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2c.—*Conversion table for percentage of standard weight*[1]*: 22-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	82	84	86	89	91	94	96	99	102	105	108	111	115
71	83	85	87	90	93	96	98	101	103	106	109	113	116
72	84	86	88	91	94	97	99	102	105	108	111	114	118
73	85	87	90	92	95	98	101	103	106	109	112	116	119
74	86	89	91	94	97	100	102	105	108	111	114	117	121
75	87	90	92	95	98	101	103	106	109	112	115	119	123
76	89	91	93	96	99	102	105	108	111	114	117	121	124
77	90	92	94	98	101	104	106	109	112	115	118	122	126
78	91	93	96	99	102	105	107	110	114	117	120	124	128
79	92	95	97	100	103	106	109	112	115	118	121	125	129
80	93	96	98	101	104	108	110	113	116	120	123	127	131
81	94	97	99	103	106	109	111	115	118	121	124	128	133
82	96	98	101	104	107	110	113	116	119	123	126	130	134
83	97	99	102	105	108	112	114	118	121	124	127	132	136
84	98	100	103	106	110	113	116	119	122	126	129	133	137
85	99	102	104	108	111	114	117	120	124	127	131	135	139
86	100	103	105	109	112	116	118	122	125	129	132	136	141
87	101	104	107	110	114	117	120	123	127	130	134	138	142
88	103	105	108	111	115	118	121	125	128	132	135	140	144
89	104	106	109	113	116	120	122	126	130	133	137	141	146
90	105	108	110	114	117	121	124	127	131	135	138	143	147
91	106	109	112	115	119	122	125	129	132	136	140	144	149
92	107	110	113	116	120	124	127	130	134	138	141	146	150
93	108	111	114	118	121	125	128	132	135	139	143	147	152
94	110	112	115	119	123	126	129	133	137	141	144	149	154
95	111	114	116	120	124	128	131	134	138	142	146	151	155
96	112	115	118	121	125	129	132	136	140	144	147	152	157
97	113	116	119	123	127	130	133	137	141	145	149	154	159
98	114	117	120	124	128	132	135	139	143	147	150	155	160
99	115	118	121	125	129	133	136	140	144	148	152	157	162
100	116	119	122	126	130	134	137	141	145	149	153	158	163
101	118	121	124	128	132	136	139	143	147	151	155	160	165
102	119	122	125	129	133	137	140	144	148	152	157	162	167
103	120	123	126	130	134	139	142	146	150	154	158	163	168
104	121	124	127	132	136	140	143	147	151	155	160	165	170
105	122	125	129	133	137	141	144	149	153	157	161	166	172
106	123	127	130	134	138	143	146	150	154	158	163	168	173
107	125	128	131	135	140	144	147	151	156	160	164	170	175
108	126	129	132	137	141	145	148	153	157	161	166	171	177
109	127	130	133	138	142	147	150	154	159	163	167	173	178
110	128	131–2	135	139	143–4	148	151–2	156	160	164–5	169	174–5	180

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2d.—*Conversion table for percentage of standard weight*[1]*: 23-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	82	84	87	89	92	95	97	100	103	105	108	112	115
71	84	86	88	91	94	96	98	101	104	107	110	113	117
72	85	87	89	92	95	98	100	103	106	108	111	115	119
73	86	88	90	93	96	99	101	104	107	110	113	117	120
74	87	89	92	94	97	100	103	106	109	111	114	118	122
75	88	90	93	96	99	102	104	107	110	113	116	120	123
76	89	92	94	97	100	103	105	108	111	114	118	121	125
77	91	93	95	98	101	104	107	110	113	116	119	123	127
78	92	94	96	100	103	106	108	111	114	117	121	125	128
79	93	95	98	101	104	107	110	113	116	119	122	126	130
80	94	96	99	102	105	108	111	114	117	120	124	128	132
81	95	98	100	103	107	110	112	116	119	122	125	129	133
82	96	99	101	105	108	111	114	117	120	123	127	131	135
83	98	100	103	106	109	113	115	118	122	125	128	132	137
84	99	101	104	107	111	114	116	120	123	126	130	134	138
85	100	102	105	108	112	115	118	121	125	128	131	136	140
86	101	104	106	110	113	117	119	123	126	129	133	137	142
87	102	105	108	111	114	118	121	124	128	131	134	139	143
88	103	106	109	112	116	119	122	125	129	132	136	140	145
89	105	107	110	114	117	121	123	127	130	134	138	142	146
90	106	108	111	115	118	122	125	128	132	135	139	144	148
91	107	110	112	116	120	123	126	130	133	137	141	145	150
92	108	111	114	117	121	125	127	131	135	138	142	147	151
93	109	112	115	119	122	126	129	133	136	140	144	148	153
94	110	113	116	120	124	127	130	134	138	141	145	150	155
95	112	114	117	121	125	129	132	135	139	143	147	152	156
96	113	116	119	122	126	130	133	137	141	144	148	153	158
97	114	117	120	124	128	131	134	138	142	146	150	155	160
98	115	118	121	125	129	133	136	140	144	147	151	156	161
99	116	119	122	126	130	134	137	141	145	149	153	158	163
100	117	120	123	127	131	135	138	142	146	150	154	159	164
101	119	122	125	129	133	137	140	144	148	152	156	161	166
102	120	123	126	130	134	138	141	145	149	153	158	163	168
103	121	124	127	131	135	140	143	147	151	155	159	164	169
104	122	125	128	133	137	141	144	148	152	156	161	166	171
105	123	126	130	134	138	142	145	150	154	158	162	167	173
106	125	128	131	135	139	144	147	151	155	159	164	169	174
107	126	129	132	136	141	145	148	152	157	161	165	171	176
108	127	130	133	138	142	146	150	154	158	162	167	172	178
109	128	131	135	139	143	148	151	155	160	164	168	174	179
110	129	132–3	136	140	145	149	152–3	157	161–2	165–6	170	175–6	181–2

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2c.—*Conversion table for percentage of standard weight*[1]*: 24-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	83	85	87	90	93	96	98	101	103	106	109	112	117
71	84	86	89	91	94	97	99	102	105	108	111	114	118
72	85	88	90	93	96	98	101	103	106	109	112	116	120
73	87	89	91	94	97	100	102	105	108	111	114	117	122
74	88	90	92	95	98	101	103	106	109	112	115	119	123
75	89	91	93	96	99	102	105	108	111	114	117	120	125
76	90	92	95	98	101	104	106	109	112	115	118	122	127
77	91	94	96	99	102	105	108	111	114	117	120	124	128
78	93	95	97	100	103	107	109	112	115	118	121	125	130
79	94	96	98	102	105	108	110	113	117	120	123	127	132
80	95	97	100	103	106	109	112	115	118	121	124	128	133
81	96	99	101	104	107	111	113	116	120	123	126	130	135
82	97	100	102	105	109	112	114	118	121	124	128	132	137
83	98	101	103	107	110	113	116	119	123	126	129	133	138
84	100	102	105	108	111	115	117	121	124	127	131	135	140
85	101	103	106	109	113	116	119	122	125	129	132	136	142
86	102	105	107	111	114	117	120	123	127	130	134	138	143
87	103	106	108	112	115	119	121	125	128	132	135	140	145
88	104	107	110	113	117	120	123	126	130	133	137	141	147
89	106	108	111	114	118	122	124	128	131	135	138	143	148
90	107	109	112	116	119	123	126	129	133	136	140	144	150
91	108	111	113	117	121	124	127	131	134	138	142	146	152
92	109	112	115	118	122	126	128	132	136	139	143	148	153
93	110	113	116	120	123	127	130	133	137	141	145	149	155
94	111	114	117	121	125	128	131	135	139	142	146	151	157
95	113	115	118	122	126	130	133	136	140	144	148	152	158
96	114	117	120	123	127	131	134	138	142	145	149	154	160
97	115	118	121	125	129	132	135	139	143	147	151	156	162
98	116	119	122	126	130	134	137	141	145	148	152	157	163
99	117	120	123	127	131	135	138	142	146	150	154	159	165
100	118	121	124	128	132	136	139	143	147	151	155	160	166
101	120	123	126	130	134	138	141	145	149	153	157	162	168
102	121	124	127	131	135	139	142	146	150	155	159	164	170
103	122	125	128	132	136	141	144	148	152	156	160	165	171
104	123	126	129	134	138	142	145	149	153	158	162	167	173
105	124	128	131	135	139	143	146	151	155	159	163	168	175
106	126	129	132	136	140	145	148	152	156	161	165	170	176
107	127	130	133	137	142	146	149	154	158	162	166	172	178
108	128	131	134	139	143	147	151	155	159	164	168	173	180
109	129	132	136	140	144	149	152	156	161	165	169	175	181
110	130	134	137	141–2	146	150	153–4	158	162–3	167	171–2	176–7	183–4

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2f.—*Conversion table for percentage of standard weight*[1]*: 25-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	84	85	87	90	93	96	98	101	104	107	110	114	118
71	85	86	89	91	94	97	100	103	106	108	112	116	120
72	86	88	90	93	96	98	101	104	107	110	114	117	121
73	87	89	91	94	97	100	103	106	109	111	115	119	123
74	89	90	92	95	98	101	104	107	110	113	117	120	125
75	90	91	93	96	99	102	105	108	111	114	118	122	126
76	91	92	95	98	101	104	107	110	113	116	120	124	128
77	92	94	96	99	102	105	108	111	114	118	121	125	130
78	93	95	97	100	103	107	110	113	116	119	123	127	132
79	95	96	98	102	105	108	111	114	117	121	125	128	133
80	96	97	100	103	106	109	112	116	119	122	126	130	135
81	97	99	101	104	107	111	114	117	120	124	128	132	137
82	98	100	102	105	109	112	115	119	122	125	129	133	138
83	99	101	103	107	110	113	117	120	123	127	131	135	140
84	100	102	105	108	111	115	118	121	125	128	132	137	142
85	102	103	106	109	113	116	119	123	126	130	134	138	143
86	103	105	107	111	114	117	121	124	128	131	136	140	145
87	104	106	108	112	115	119	122	126	129	133	137	141	147
88	105	107	110	113	117	120	124	127	131	134	139	143	148
89	106	108	111	114	118	122	125	129	132	136	140	145	150
90	108	109	112	116	119	123	126	130	134	137	142	146	152
91	109	111	113	117	121	124	128	132	135	139	143	148	153
92	110	112	115	118	122	126	129	133	137	140	145	150	155
93	111	113	116	120	123	127	131	134	138	142	147	151	157
94	112	114	117	121	125	128	132	136	140	143	148	153	158
95	114	115	118	122	126	130	133	137	141	145	150	154	160
96	115	117	120	123	127	131	135	139	143	146	151	156	162
97	116	118	121	125	129	132	136	140	144	148	153	158	163
98	117	119	122	126	130	134	138	142	146	149	154	159	165
99	118	120	123	127	131	135	139	143	147	151	156	161	167
100	119	121	124	128	132	136	140	144	148	152	157	162	168
101	121	123	126	130	134	138	142	146	150	154	159	164	170
102	122	124	127	131	135	139	143	147	151	156	161	166	172
103	123	125	128	132	136	141	145	149	153	157	162	167	174
104	124	126	129	134	138	142	146	150	154	159	164	169	175
105	125	128	131	135	139	143	147	152	156	160	165	171	177
106	127	129	132	136	140	145	149	153	157	162	167	172	179
107	128	130	133	137	142	146	150	155	159	163	168	174	180
108	129	131	134	139	143	147	152	156	160	165	170	175	182
109	130	132	136	140	144	149	153	157	162	166	172	177	184
110	131–2	134	137	141–2	146	150	154–5	159	163–4	168	173–4	179	185–6

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2g.—*Conversion table for percentage of standard weight*[1]*: 26-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	84	86	88	91	94	96	99	102	105	108	111	115	119
71	86	87	89	92	95	98	101	103	106	109	113	116	120
72	87	88	90	93	96	99	102	105	108	111	114	118	122
73	88	90	92	95	98	101	103	106	109	112	116	119	124
74	89	91	93	96	99	102	105	108	111	114	117	121	126
75	90	92	94	97	100	103	106	109	112	115	119	123	127
76	92	93	95	99	102	105	108	111	114	117	121	124	129
77	93	94	97	100	103	106	109	112	115	118	122	126	131
78	94	96	98	101	104	107	110	114	117	120	124	128	132
79	95	97	99	102	106	109	112	115	118	121	125	129	134
80	96	98	100	104	107	110	113	116	120	123	127	131	136
81	98	99	102	105	108	111	115	118	121	124	128	133	137
82	99	101	103	106	110	113	116	119	123	126	130	134	139
83	100	102	104	108	111	114	118	121	124	127	132	136	141
84	101	103	105	109	112	116	119	122	126	129	133	137	142
85	102	104	107	110	114	117	120	124	127	131	135	139	144
86	104	105	108	111	115	118	122	125	129	132	136	141	146
87	105	107	109	113	116	120	123	127	130	134	138	142	148
88	106	108	110	114	118	121	125	128	132	135	140	144	149
89	107	109	112	115	119	122	126	130	133	137	141	146	151
90	108	110	113	117	120	124	127	131	135	138	143	147	153
91	110	112	114	118	122	125	129	132	136	140	144	149	154
92	111	113	115	119	123	127	130	134	138	141	146	150	156
93	112	114	117	120	124	128	132	135	139	143	147	152	158
94	113	115	118	122	126	129	133	137	141	144	149	154	159
95	114	116	119	123	127	131	134	138	142	146	151	155	161
96	116	118	120	124	128	132	136	140	144	147	152	157	163
97	117	119	122	126	130	133	137	141	145	149	154	159	164
98	118	120	123	127	131	135	139	143	147	150	155	160	166
99	119	121	124	128	132	136	140	144	148	152	157	162	168
100	120	122	125	129	133	137	141	145	149	153	158	163	169
101	122	124	127	131	135	139	143	147	151	155	160	165	171
102	123	125	128	132	136	140	144	148	152	157	162	167	173
103	124	126	129	133	137	142	146	150	154	158	163	168	175
104	125	127	130	135	139	143	147	151	155	160	165	170	176
105	126	129	132	136	140	144	149	153	157	161	166	172	178
106	128	130	133	137	141	146	150	154	158	163	168	173	180
107	129	131	134	139	143	147	151	156	160	164	170	175	181
108	130	132	135	140	144	148	153	157	161	166	171	177	183
109	131	133	137	141	145	150	154	159	163	167	173	178	185
110	132–3	135	138	142–3	147	151–2	156	160	164–5	169	174–5	180	186–7

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2h.—*Conversion table for percentage of standard weight*[1]*: 27-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	85	87	89	91	94	96	99	102	105	108	111	115	119
71	86	88	90	92	95	98	101	103	106	109	113	117	121
72	88	89	91	93	96	99	102	105	108	111	114	119	123
73	89	90	92	95	98	101	103	106	109	112	116	120	125
74	90	92	94	96	99	102	105	108	111	114	117	122	126
75	91	93	95	97	100	103	106	109	112	115	119	123	128
76	92	94	96	99	102	105	108	111	114	117	121	125	130
77	94	95	98	100	103	106	109	112	115	118	122	127	131
78	95	96	99	101	104	107	110	114	117	120	124	128	133
79	96	98	100	102	106	109	112	115	118	121	125	130	135
80	97	99	101	104	107	110	113	116	120	123	127	132	136
81	99	100	103	105	108	111	115	118	121	124	128	133	138
82	100	101	104	106	110	113	116	119	123	126	130	135	140
83	101	103	105	108	111	114	118	121	124	127	132	137	142
84	102	104	106	109	112	116	119	122	126	129	133	138	143
85	103	105	108	110	114	117	120	124	127	131	135	140	145
86	105	106	109	111	115	118	122	125	129	132	136	142	147
87	106	108	110	113	116	120	123	127	130	134	138	143	148
88	107	109	111	114	118	121	125	128	132	135	140	145	150
89	108	110	113	115	119	122	126	130	133	137	141	146	152
90	109	111	114	117	120	124	127	131	135	138	143	148	153
91	111	112	115	118	122	125	129	132	136	140	144	150	155
92	112	114	116	119	123	127	130	134	138	141	146	151	157
93	113	115	118	120	124	128	132	135	139	143	147	153	159
94	114	116	119	122	126	129	133	137	141	144	149	155	160
95	115	117	120	123	127	131	134	138	142	146	151	156	162
96	117	119	121	124	128	132	136	140	144	147	152	158	164
97	118	120	123	126	130	133	137	141	145	149	154	160	165
98	119	121	124	127	131	135	139	143	147	150	155	161	167
99	120	122	125	128	132	136	140	144	148	152	157	163	169
100	121	123	126	129	133	137	141	145	149	153	158	164	170
101	123	125	128	131	135	139	143	147	151	155	160	166	172
102	124	126	129	132	136	140	144	148	152	157	162	168	174
103	125	127	130	133	137	142	146	150	154	158	163	169	176
104	126	128	132	135	139	143	147	151	155	160	165	171	177
105	128	130	133	136	140	144	149	153	157	161	166	173	179
106	129	131	134	137	141	146	150	154	158	163	168	174	181
107	130	132	135	139	143	147	151	156	160	164	170	176	182
108	131	133	137	140	144	148	153	157	161	166	171	178	184
109	132	135	138	141	145	150	154	159	163	167	173	179	186
110	134	136	139	142–3	147	151–2	156	160	164–5	169	174–5	181–2	187–8

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 21.—*Conversion table for percentage of standard weight*[1]*: 28-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	86	87	89	91	94	97	100	103	105	108	112	116	120
71	87	89	91	93	96	98	101	104	107	110	113	118	122
72	88	90	92	94	97	100	103	106	108	111	115	119	124
73	90	91	93	95	98	101	104	107	110	113	117	121	125
74	91	92	94	97	100	103	106	109	111	114	118	123	127
75	92	93	96	98	101	104	107	110	113	116	120	124	129
76	93	95	97	99	102	105	108	111	114	118	121	126	130
77	94	96	98	101	104	107	110	113	116	119	123	128	132
78	96	97	100	102	105	108	111	114	117	121	125	129	134
79	97	98	101	103	106	110	113	116	119	122	126	131	136
80	98	100	102	104	108	111	114	117	120	124	128	132	137
81	99	101	103	106	109	112	116	119	122	125	129	134	139
82	101	102	105	107	110	114	117	120	123	127	131	136	141
83	102	103	106	108	112	115	118	122	125	128	132	137	142
84	103	105	107	110	113	116	120	123	126	130	134	139	144
85	104	106	108	111	114	118	121	125	128	131	136	141	146
86	105	107	110	112	116	119	123	126	129	133	137	142	148
87	107	108	111	114	117	121	124	128	131	134	139	144	149
88	108	110	112	115	118	122	125	129	132	136	140	146	151
89	109	111	114	116	120	123	127	130	134	138	142	147	153
90	110	112	115	117	121	125	128	132	135	139	144	149	154
91	112	113	116	119	122	126	130	133	137	141	145	151	156
92	113	115	117	120	124	127	131	135	138	142	147	152	158
93	114	116	119	121	125	129	133	136	140	144	148	154	160
94	115	117	120	123	126	130	134	138	141	145	150	156	161
95	116	118	121	124	128	132	135	139	143	147	152	157	163
96	118	120	122	125	129	133	137	141	144	148	153	159	165
97	119	121	124	127	130	134	138	142	146	150	155	161	166
98	120	122	125	128	132	136	140	144	147	151	156	162	168
99	121	123	126	129	133	137	141	145	149	153	158	164	170
100	122	124	127	130	134	138	142	146	150	154	159	165	171
101	124	126	129	132	136	140	144	148	152	156	161	167	173
102	125	127	130	133	137	141	145	149	153	158	163	169	175
103	126	128	131	134	139	143	147	151	155	159	164	170	177
104	127	129	133	136	140	144	148	152	156	161	166	172	178
105	129	131	134	137	141	145	150	154	158	162	167	174	180
106	130	132	135	138	143	147	151	155	159	164	169	175	182
107	131	133	136	140	144	148	152	157	161	165	171	177	183
108	132	134	138	141	145	150	154	158	162	167	172	179	185
109	133	136	139	142	147	151	155	160	164	168	174	180	187
110	135	137	140	143–4	148	152–3	157	161–2	165–6	170	175–6	182–3	189

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2j.—*Conversion table for percentage of standard weight*[1]*: 29-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	87	88	90	92	95	98	101	103	106	109	112	117	121
71	88	89	91	94	96	99	102	105	108	111	114	118	123
72	89	90	93	95	98	101	103	106	109	112	116	120	124
73	90	92	94	96	99	102	105	108	111	114	117	122	126
74	92	93	95	97	100	103	106	109	112	115	119	123	128
75	93	94	96	99	102	105	108	111	114	117	120	125	129
76	94	95	98	100	103	106	109	112	115	118	122	127	131
77	95	97	99	101	104	108	111	114	117	120	124	128	133
78	96	98	100	103	106	109	112	115	118	121	125	130	135
79	98	99	102	104	107	110	113	117	120	123	127	132	136
80	99	100	103	105	108	112	115	118	121	124	128	133	138
81	100	102	104	107	110	113	116	120	123	126	130	135	140
82	101	103	105	108	111	114	118	121	124	128	132	137	142
83	103	104	107	109	113	116	119	123	126	129	133	138	143
84	104	105	108	111	114	117	121	124	127	131	135	140	145
85	105	107	109	112	115	119	122	125	129	132	136	142	147
86	106	108	111	113	117	120	123	127	130	134	138	143	148
87	108	109	112	114	118	121	125	128	132	135	140	145	150
88	109	110	113	116	119	123	126	130	133	137	141	147	152
89	110	112	114	117	121	124	128	131	135	138	143	148	154
90	111	113	116	118	122	126	129	133	136	140	144	150	155
91	112	114	117	120	123	127	131	134	138	142	146	152	157
92	114	115	118	121	125	128	132	136	139	143	148	153	159
93	115	117	120	122	126	130	133	137	141	145	149	155	160
94	116	118	121	124	127	131	135	139	142	146	151	157	162
95	117	119	122	125	129	133	136	140	144	148	152	158	164
96	119	120	123	126	130	134	138	142	145	149	154	160	166
97	120	122	125	128	131	135	139	143	147	151	156	162	167
98	121	123	126	129	133	137	141	145	148	152	157	163	169
99	122	124	127	130	134	138	142	146	150	154	159	165	171
100	123	125	128	131	135	139	143	147	151	155	160	166	172
101	125	127	130	133	137	141	145	149	153	157	162	168	174
102	126	128	131	134	138	142	146	150	155	159	164	170	176
103	127	129	132	135	140	144	148	152	156	160	165	171	178
104	128	130	134	137	141	145	149	153	158	162	167	173	179
105	130	132	135	138	142	146	151	155	159	163	168	175	181
106	131	133	136	139	144	148	152	156	161	165	170	176	183
107	132	134	137	141	145	149	154	158	162	166	172	178	185
108	133	135	139	142	146	151	155	159	164	168	173	180	186
109	135	137	140	143	148	152	156	161	165	169	175	181	188
110	136	138	141–2	145	149	153–4	158	162–3	167	171–2	176–7	183–4	190

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

TABLE 2k.—*Conversion table for percentage of standard weight*[1]*: 30-year-old males*

[In pounds]

Standard weight (Percent)	Height in inches—												
	60	61	62	63	64	65	66	67	68	69	70	71	72
70	87	88	90	92	95	98	101	103	106	110	113	117	122
71	88	89	91	94	96	99	102	105	108	111	115	119	123
72	89	90	93	95	98	101	103	106	109	113	116	121	125
73	90	92	94	96	99	102	105	108	111	114	118	122	127
74	92	93	95	97	100	103	106	109	112	116	120	124	129
75	93	94	96	99	102	105	108	111	114	117	121	126	130
76	94	95	98	100	103	106	109	112	115	119	123	127	132
77	95	97	99	101	104	108	111	114	117	121	124	129	134
78	96	98	100	103	106	109	112	115	118	122	126	131	135
79	98	99	102	104	107	110	113	117	120	124	128	132	137
80	99	100	103	105	108	112	115	118	121	125	129	134	139
81	100	102	104	107	110	113	116	120	123	127	131	136	141
82	101	103	105	108	111	114	118	121	124	128	133	137	142
83	103	104	107	109	113	116	119	123	126	130	134	139	144
84	104	105	108	111	114	117	121	124	127	132	136	141	146
85	105	107	109	112	115	119	122	125	129	133	137	142	148
86	106	108	111	113	117	120	123	127	130	135	139	144	149
87	108	109	112	114	118	121	125	128	132	136	141	146	151
88	109	110	113	116	119	123	126	130	133	138	142	147	153
89	110	112	114	117	121	124	128	131	135	139	144	149	154
90	111	113	116	118	122	126	129	133	136	141	145	151	156
91	112	114	117	120	123	127	131	134	138	142	147	152	158
92	114	115	118	121	125	128	132	136	139	144	149	154	160
93	115	117	120	122	126	130	133	137	141	146	150	156	161
94	116	118	121	124	127	131	135	139	142	147	152	157	163
95	117	119	122	125	129	133	136	140	144	149	153	159	165
96	119	120	123	126	130	134	138	142	145	150	155	161	167
97	120	122	125	128	131	135	139	143	147	152	156	162	168
98	121	123	126	129	133	137	141	145	148	153	158	164	170
99	122	124	127	130	134	138	142	146	150	155	160	166	172
100	123	125	128	131	135	139	143	147	151	156	161	167	173
101	125	127	130	133	137	141	145	149	153	158	163	169	175
102	126	128	131	134	138	142	146	150	155	160	165	171	177
103	127	129	132	135	140	144	148	152	156	161	166	173	179
104	128	130	134	137	141	145	149	153	158	163	168	174	180
105	130	132	135	138	142	146	151	155	159	164	170	176	182
106	131	133	136	139	144	148	152	156	161	166	171	178	184
107	132	134	137	141	145	149	154	158	162	167	173	179	186
108	133	135	139	142	146	151	155	159	164	169	174	181	187
109	135	137	140	143	148	152	156	161	165	171	176	183	189
110	136	138	141–2	145	149	153–4	158	162–3	167	172–3	178	184–5	191–2

[1] Calculated from adjusted (to nude weight, no shoes) Medico-Actuarial tables. If a weight within the range listed is not present, use next lower pound to calculate percentage.

IV. Instructions for Coding, Punching, and Sorting McBee Cards

The purpose of using McBee edge-punched cards in the recording of data is to facilitate a rapid, current compilation of data for guidance in the execution of the survey, and for immediate preparation of preliminary reports of findings.

In the preceding section, a description has been given of the identification data and physical findings which are to be recorded and the McBee hole numbers which correspond to the items.

Holes 1–12 on the card are available for numeric punching of the first three digits of the four-digit card number. Holes 13–16 and 17–20 are for numeric punching of location and activity data, according to codes which can be set up as found to be appropriate in each survey.

Holes 21–24 are for time-in-service, which can be coded as follows:

1. 0 month to 3 months.
2. 3 months to 6 months.
3. 6 months to 1 year.
4. 1 year to 2 years.
5. 2 years to 3 years.
6. 3 years to 4 years.
7. 4 years to 5 years.
8. 5 years to 10 years.
9. 10 years to 15 years.
10. 15 years and over.

Holes 25–28 are for percent of standard weight, which can be coded as follows:

1. Below 60%.
2. 60–69.
3. 70–79.
4. 80–89.
5. 90–99.
6. 100–109.
7. 110–119.
8. 120–129.
9. 130–139.
10. 140% and above.

Hole 29 is for diarrhea. If this is recorded by the physician, the hole will be marked and punched. A card without hole 29 punched out will then be one for an individual without a finding of diarrhea. (It is essential that all items be examined for as no provision is made in this system of punching for missing data.)

Holes 30–31 are for general appearance.

Good: Leave unpunched.
Fair: Punch 30.
Poor: Punch 31.
Cachexia: Punch 30 and 31.

Clinical findings are recorded in holes 32–96, according to the system of punching as described for diarrhea: nonpunching means the condition was absent; a punch identifies a positive finding.

For clinical items which provide for classification according to severity of condition or indication of type of condition present, the outer box beside the item will be marked, in addition to the marking of the inner box. For the McBee punching of these, any finding will be punched without regard to the further classification. (An exception to the procedure of marking the outer box beside a positive finding occurs for the classification of areas of follicular keratosis, item 73. A finding of follicular keratosis in item 72 necessarily implies its presence in at least one area; for item 73, then, the outer box is to be marked only if multiple areas are involved, in which case it will be punched.)

Holes 97–99 are for examiner code numbers, to identify the clinician making the examination. Punching can be as follows:

Examiner No. 1: Punch 97.
Examiner No. 2: Punch 98.
Examiner No. 3: Punch 99.
Examiner No. 4: Punch 97 and 98.
Examiner No. 5: Punch 97 and 99.
Examiner No. 6: Punch 98 and 99.

If needed, nonpunching in this section can be taken to indicate one of the examiners. If only one clinician does all examinations, there is obviously no need to punch this section.

Holes 100 and 101 are provided for punching to identify individuals who have blood or urine samples taken.

Holes 102–137 are for the recording of the biochemical measurements, in numeric codes. Suggested coding-intervals for the McBee card coding of the various measurements are given in table 3.

For most of the clinical information, the use of the card for tabulation of results is simple. For findings which are present, the box adjacent to the finding will be marked by the clinician; for punching, the numbers on the edge of the card corresponding to positive findings are marked with a red pencil. It is recommended that the marking be carried out on all of a series of cards being processed, before any punching is done. The marking should be carefully checked, by reexamination of the marked set of cards, for errors of commission and omission.

The numeric punching involves the use of four holes to record any digit from 1–10 with a maximum of two holes punched. The four holes allotted to an item have printed numbers 1, 2, 4 and 7 beside them. To record a number, holes are punched out according to the following system:

Code 1: Punch 1.	Code 6: Punch 4 and 2.
Code 2: Punch 2.	Code 7: Punch 7.
Code 3: Punch 2 and 1.	Code 8: Punch 7 and 1.
Code 4: Punch 4.	Code 9: Punch 7 and 2.
Code 5: Punch 4 and 1.	Code 10: Punch 7 and 4.

The absence of any punches will indicate that no data were obtained.

Example.—An individual has been in the Service for 2 years, 3 months. Code 5 is assigned and entered in the space provided at the top of the card. Holes 1, and 4, in the time-in-service section, on the edge of the card, are marked.

Then, using a hand-operated punch, the holes indicated by the marks are punched out. This punching operation should also be checked for accuracy. If mistakes are made in the punching, they should be repaired with gummed perforated "card saver" strips.

To determine for a group of individuals whose cards have been punched, the number who had a positive finding on any given item, insert a rod through the hole corresponding to the item. Suspend and shake the cards; all those with a positive finding will drop from the pile of cards and can then be counted. A McBee Company "needle" will be available for this operation; knitting needles make good substitutes. For the sorting operation, all cards have to be oriented alike. The upper left-hand corner of the front of each card is clipped, so that proper orientation can be checked while making sorts by inspection of this corner.

In sorting on coded data which have been punched into the four-hole sequence, first sort on the No. 7 hole, and remove those punched on No. 7; then sort successive remainders on the Nos. 4, 2, and 1 holes. The five piles of cards resulting are then further sorted on the lower hole numbers, giving codes 10, 9, 8, and 7 from the first pile, etc.

V. Instructions for Coding for I.B.M. Card Analysis for Detailed Physical Examinations

Space is provided at the bottom of each side of the McBee card for the coding of data for subsequent detailed analysis of results, using International Business Machine (I.B.M.) equipment. IT IS ESSENTIAL THAT THE NUMBERS ENTERED IN THESE SPACES BE LEGIBLE.

Analysis of the clinical findings of the survey can be markedly speeded by preparation of the I.B.M. coding while in the field, if personnel and available slack-time allow. An intelligent clerk should be able to do the coding, but accuracy is essential and any delegation of this work to an assistant should be subject to regular and frequent checking.

The I.B.M. punch card provides 80 columns for the recording of information. Each column provides 12 spaces for coding, numbered 0–9, plus two other spaces usually designated X and Y. An I.B.M. code has been set up which utilizes columns 1–79. On the McBee card, and in section III, the McBee items corresponding to each I.B.M. column are indicated.

The I.B.M. code, in outline, is as follows:

I.B.M. Column

1–4 Enter the four-digit card number.

5–7 Enter location, activity, and time-in-service, according to the numeric codes as used for the McBee punching.

8 Enter area-of-origin according to a numeric code of not more than 11 categories.

9 Enter rural-urban origin as: 0 = Rural; 1 = Urban.

10–12 For the direct recording of a three-digit percent-of-standard-weight. In preparing for the punching of the I.B.M. cards, the slide rule calculations, as done in the field for the McBee coding, will later be verified for accuracy by machine calculation.

13–14 Enter age.

15–16 Enter height.

17–19 Enter weight.

20 Enter suspected disease according to the code:

0 = None of three	4 = Malaria + Trachoma
1 = Malaria	5 = Malaria + TBC
2 = Trachoma	6 = Trachoma + TBC
3 = TBC	7 = All three.

If "Other" diseases are frequently recorded, the information can be utilized by a modification of the code, either in the field or subsequently. If no disease history is taken, code as "Y".

21 Enter current disease according to the code:

0 = None 1 = Diarrhea

Comments for I.B.M. column 20 also apply here.

22 General appearance:

0 = Good 1 = Fair 2 = Poor 3 = Cachexia

23 Hair:

0 = No finding
1 = Staring (McBee No. 32)
2 = Depigmentation (McBee No. 33)
3 = Both of these (McBee Nos. 32, 33)

24 Glands:

Code McBee items Nos. 34, 35, and 36, in a 0–7 coding system as used in I.B.M. column 20, e.g.:

0 = None of three	4 = McBee Nos. 34 and 35
1 = Thyroid (McBee No. 34)	5 = McBee Nos. 34 and 36
2 = Parotid (McBee No. 35)	6 = McBee Nos. 35 and 36
3 = Submax. (McBee No. 36)	7 = All three.

25 Skin (Face and neck). Seborrhea:

Code McBee items Nos. 37 and 38 in the 0–3 coding system as used in I.B.M. column 23.

26 Skin (Face and neck). Erythema and pigmentation:

Code McBee items Nos. 39 and 40 in the 0–3 coding system as used in I.B.M. column 23.

I.B.M. Column

27 Eyes (Thickened Conjunctivae). McBee No. 41:
0 = Negative 1 = Grade I 2 = Grade II

28 Eyes. McBee items Nos. 42, 43, 44:
Code in the 0–7 coding system as used in I.B.M. column 24.

29 Eyes. McBee items Nos. 45, 46, 47:
Code in the 0–7 coding system as used in I.B.M. column 24.

30 Lips. McBee items Nos. 48, 49, 50:
Code in the 0–7 coding system as used in I.B.M. column 24.

31 Tongue. McBee items Nos. 51, 52:
0 = Neither finding
1 = Filiform papillary atrophy, slight
2 = Filiform papillary atrophy, moderate
3 = Filiform papillary atrophy, severe
4 = Fungiform papillary atrophy
5 = Findings of 4 + 1
6 = Findings of 4 + 2
7 = Findings of 4 + 3

32 Tongue. McBee items Nos. 53, 54:
Coding follows the same sequence as used in I.B.M. column 31.

33 Tongue. McBee items Nos. 55, 56, 57:
Code in the 0–7 coding system as used in I.B.M. column 24.

34 Tongue. McBee items Nos. 58, 59, 60:
Code in the 0–7 coding system as used in I.B.M. column 24.

35 Gums. McBee items Nos. 61, 62, 63:
Code in the 0–7 coding system as used in I.B.M. column 24.

36 Gums. McBee items Nos. 64, 65, 66:
Code in the 0–7 coding system as used in I.B.M. column 24.

37 Teeth (Unfilled Caries). McBee No. 67:
0 = None 2 = 3 or 4 teeth
1 = 1 or 2 teeth 3 = 5 or more teeth

38 Teeth (Filled Caries). McBee No. 68:
0 = None 2 = 3 or 4 teeth
1 = 1 or 2 teeth 3 = 5 or more teeth

39 Teeth (Edentulous). McBee No. 69:
0 = Not 1 = With plates 2 = Without plates

40 Teeth. McBee items Nos. 70 and 71 + Malposition:
Code in the 0–7 coding system as used in I.B.M. column 24.

41 Skin (General). Follicular Keratosis. McBee No. 72:
0 = Negative 1 = Grade I 2 = Grade II

42 Areas of follicular keratosis. McBee No. 73 a-c:
Code in the 0–7 coding system as used in I.B.M. column 24.

43 Areas of follicular keratosis. McBee No. 73 d-e:
Code in the 0–3 coding system as used in I.B.M. column 23.

44 Skin (General). McBee items Nos. 74, 75, 76:
Code in the 0–7 coding system as used in I.B.M. column 24.

45 Skin (General). McBee items Nos. 77, 78, 79:
Code in the 0–7 coding system as used in I.B.M. column 24.

46 Skin (General). McBee items Nos. 80, 81, 82:
Code in the 0–7 coding system as used in I.B.M. column 24.

47 Abdomen. McBee items Nos. 83, 84, 85:
Code in the 0–7 coding system as used in I.B.M. column 24.

48 Lower extremities. McBee items Nos. 86, 87, 88:
Code in the 0–7 coding system as used in I.B.M. column 24.

49 Lower extremities. McBee items Nos. 89, 90:
Coding follows the same sequence as used in I.B.M. column 31.

I.B.M. Column	
50	Lower extremities. McBee items Nos. 91, 92, 93: Code in the 0–7 coding system as used in I.B.M. column 24.
51–56	Enter blood pressure in full. Systolic followed by diastolic, each as three-digit numbers: e.g., 150110, 120080, 090060.
57–58	Enter pulse rate as the last two digits of the count; e.g., pulse 84 is entered as 84, pulse 60 is entered as 60, but pulse 120 is entered as 20.
59–60	Enter arm skin measurement in mm.
61–62	Enter scapula skin measurement in mm.
63	Enter examiner code number, according to numeric code used for the McBee punching. If only one clinician does all the examinations of the survey, this column can be left blank, for possible use in recording other data.

Column 63 completes the recording of the clinical examination. Columns 64–79 are for recording of biochemical data; these results can be recorded in the field only insofar as the laboratory results are completed at that time; this recording will only involve the fewer numbers of individuals sampled for biochemical measurement.

For the blood measurements, two I.B.M. columns are provided for each item (with the exception of plasma protein), to allow more detailed tabulations than can be made using the one-column system of coding for the McBee card. Coding systems as suggested below are contingent upon their appropriateness according to the general level of values found in any survey area.

I.B.M. Column	
64	*Total plasma protein.*—Use same numeric code as selected for the McBee punching.
65–66	*Hemoglobin.*—If few or no determinations are below 10.0, then code the grams and tenths of grams by which a measurement exceeds 10.0, e.g., for 13.6, code 3 in column 65 and 6 in column 66. For a value of, say, 9.1, code 9 and X in column 65, and 1 in column 66. (The "X" to be double-punched in column 65 distinguishes the coded value from 19.1.)
67–68	*Hematocrit.*—Code the percent value of the determination, discarding fractions, e.g., code 46.5 with 4 in column 67, and 6 in column 68.
69–70	*Mean cell hemoglobin concentration.*—In calculation, record to 0.1 percent, discarding remainders, e.g., record 32.86 as 32.8. Code the percent value of the determination, e.g., code 32.8 with 3 in column 69 and 2 in column 70.
71–72	*Serum vitamin C.*—Code milligrams and tenths, discarding hundredths, e.g., code 1.27 with 1 in column 71 and 2 in column 72. Code 0.21 with 0 in column 71 and 2 in column 72.
73–74	*Serum vitamin A.*—Code micrograms, in tens and units, discarding fractions, e.g., code 28.7 with 2 in column 73 and 8 in column 74. Code 08.3 with 0 in column 73 and 8 in column 74.
75–76	*Serum carotene.*—Code micrograms, in hundreds and tens, discarding units and fractions, e.g., code 128.3 with 1 in column 75 and 2 in column 76. Code 61.9 with 0 in column 75 and 6 in column 76.
77	*Thiamine excretion.*—Use same numeric code as selected for the McBee punching.
78	*Riboflavin excretion.*—Use same numeric code as selected for the McBee punching.
79	*N'Methylnicotinamide excretion.*—Use same numeric code as selected for the McBee punching.

TABLE 3.—*Suggested coding-intervals for McBee-coding of biochemical data*

Code No.	T.P.P. gm/100 ml	Hemoglobin gm/100 ml	Hematocrit (percent)	M.C.H.C. (percent)	Serum vitamin C mg/100 ml
1	<5.0	<10.0	<30	<20.0	<0.10
2	5.0–5.4	10.0–10.9	30–32	20.0–21.9	0.10–0.19
3	5.5–5.8	11.0–11.9	33–35	22.0–23.9	0.20–0.29
4	5.9–6.1	12.0–12.9	36–38	24.0–25.9	0.30–0.39
5	6.2–6.5	13.0–13.9	39–41	26.0–27.9	0.40–0.59
6	6.6–6.9	14.0–14.9	42–44	28.0–29.9	0.60–0.79
7	7.0–7.3	15.0–15.9	45–47	30.0–31.9	0.80–0.99
8	7.4–7.6	16.0–16.9	48–50	32.0–33.9	1.00–1.19
9	7.7–8.0	17.0–17.9	51–53	34.0–35.9	1.20–1.39
10	>8.0	>17.9	>53	>35.9	>1.39

Code No.	Serum vitamin A mcg/100 ml	Serum carotene mcg/100 ml	Thiamine mcg/6 hr.	Riboflavin mcg/6 hr.	N′ Methylnic. mg/6 hr.
1	<10	<10	<10	<10	<0.2
2	10–14	10–19	10–24	10–19	0.2–0.5
3	15–19	20–29	25–49	20–29	0.6–0.9
4	20–24	30–39	50–74	30–39	1.0–1.5
5	25–29	40–59	75–99	40–59	1.6–1.9
6	30–34	60–79	100–124	60–79	2.0–2.9
7	35–39	80–99	125–149	80–99	3.0–3.9
8	40–44	100–119	150–199	100–149	4.0–4.9
9	45–49	120–139	200–249	150–199	5.0–5.9
10	>49	>139	>249	>199	>5.9

VI. Instructions for Coding of I.B.M. Card Analysis for Abbreviated Physical Examination Card

Space is provided at the bottom of the card for the coding of data for subsequent detailed analyses of the results, using I.B.M. equipment. IT IS ESSENTIAL THAT THE NUMBERS ENTERED IN THE SPACE BE LEGIBLE.

The I. B. M. code, in outline, is as follows:

I.B.M. Column

1–5 Enter the five digit card number.

6–9 Enter location, activity, time in service and area of origin according to the numeric codes chosen for the I.B.M. card of the detailed examination.

10 Enter rural-urban origin as: 0 = Rural; 1 = Urban.

11–12 Enter age.

13–14 Enter height.

15–17 Enter weight.

18–20 Enter standard weight.

21–23 For the direct recording of a three-digit percentage of standard weight. LEAVE BLANK. This value will be computed by machine.

24 Glands: Enter 0 for negative finding; 1 if thyroid enlarged.

25 Skin—Face and neck: Enter 0 for negative finding; 1 for nasolabial seborrhea.

26 Eyes: Enter 0 for negative finding; 1 for Bitot's spots.

27 Lips:

0 = None of three
1 = Angular scars only
2 = Cheilosis only
3 = Angular scars and cheilosis
4 = Angular lesions only
5 = Angular lesions and angular scars
6 = Angular lesions and cheilosis
7 = All three

I.B.M. Column	
28	Tongue, magenta colored and filiform papillary atrophy: 0 = Neither finding 1 = Filiform papillary atrophy, slight 2 = Filiform papillary atrophy, moderate 3 = Filiform papillary atrophy, severe 4 = Magenta colored 5 = Findings 4 + 1 6 = Findings 4 + 2 7 = Findings 4 + 3
29	Tongue, Glossitis: Enter 0 for negative finding; 1 for glossitis.
30	Gums: Enter 0 for negative findings; 1 for scorbutic type.
31	Skin—General, follicular keratosis: Enter 0 if negative finding, 1 for arms only, 2 for back only and 3 for both arms and back.
32	Skin—General, scrotal dermatitis and pellagrous lesions: 0 = Neither finding 1 = Pellagrous lesions only 2 = Scrotal dermatitis only 3 = Both pellagrous lesions and scrotal dermatitis
33	Lower Extremities, Bilateral Edema and Loss of Ankle Jerk: 0 = Neither finding 1 = Loss of ankle jerk on right side 2 = Loss of ankle jerk on left side 3 = Loss of ankle jerk on both sides 4 = Bilateral edema 5 = Findings of 4 + 1 6 = Findings of 4 + 2 7 = Findings of 4 + 3
34	Lower Extremities, calf tenderness: 0 = Negative finding 1 = Slight tenderness 2 = Moderate tenderness 3 = Severe tenderness
35	Studied further: Enter 0 if not studied further; 1 if studied further.
36–39	Enter the four digit card number of the McBee card used for special detailed clinical examination.

Biochemical Methods

I. Introduction

The biochemical studies together with the data of the physical examinations provide a means for estimating the proportion of the population in various broad zones of nutriture. When considered together with the physical examinations and dietary data, the biochemical studies, including saturation tests, enable a more definitive appraisal of the nutritional status of individuals and populations.

The equipment and supply list given in chapter 10 has been compiled so as to supply all essentials necessary for the prescribed and elective biochemical methods for blood and urine analysis. Sufficient equipment and supplies have been included to allow completing of the prescribed analyses on approximately 100 blood and 100 urine samples per week. Sufficient chemicals have been listed to permit analysis of approximately 3,000 samples. For most surveys a total of about 500 blood and 500 urine samples will be analyzed; however the actual number of determinations will depend upon the overall sampling of the population surveyed. The excess supply of chemicals and expendable equipment has been purposely included so as to enable the host country to continue a clinical biochemical laboratory.

1. Validity of Analytical Methods and Recoveries

Although the methods described in this manual have been selected for their relative reliability, among other things, the experienced chemist knows that any analytical procedure is subject to numerous possible errors. Consequently, the laboratory supervisor should routinely check all methods to be certain of valid results. Once a method has been established the continuation of results of similar magnitude will usually suffice to indicate validity. However, in nutrition surveys where wide variations, or even consistent abnormal values, may result, it is not possible to judge a method by the "appearance" of results.

Due to time limitations, it often will not be possible to perform thorough recovery experiments on all procedures. Probably the simplest test of validity is to check all methods by using blood and urine specimens from normal, healthy individuals. For this purpose it is suggested that after the laboratory has been set up, a trial run on 6 to 10 normal men be performed. The individuals selected should be well-fed by American standards of diet. These may be the United States team personnel, native officer personnel, professional class civilians, etc. If the results fall in the normal ranges indicated below, one may be reasonably certain that the methods have been performed correctly.

If time permits, or if results obtained appear questionable, recovery experiments should be carried out. For this purpose, aliquots of a urine or serum sample are run with added amounts of the substance in question. Usually two levels of added material should suffice. The amounts added should be about one-half and equal to the amount in the aliquot taken (assume a normal concentration in the specimen).

Do not add quantities which are several times greater than the amount in the aliquot, as this may give a false indication of the accuracy of the method. For plasma vitamin C, and urinary vitamin C, riboflavin and thiamine it is advisable to determine the recoveries from the field collection time so as to establish whether temperature, or elapsed time between collection and analysis has caused destruction of the vitamin in question. Thus provision should be made to divide numerous samples, properly labelled for recovery determination. Ascorbic acid for recovery experiments should be made up in the "acid filtrate" to avoid the deterioration which occurs in aqueous solution.

Ranges of Values Found in Healthy United States Adult Males [1]

(1) Total serum protein: 6.5–8.2 gm/100 ml.
(2) Hemoglobin: 14.5–17.0 gm/100 ml.
(3) Hematocrit: 44–50.
(4) Plasma vitamin C: 0.4–1.0 mg/100 ml.
(5) Plasma vitamin A: 25–70 mcg/100 ml.
(6) Plasma carotene: 40–150 mcg/100 ml.
(7) Urine thiamine: 30–100 mcg/6 hr.
(8) Urine riboflavin: 50–200 mcg/6 hr.
(9) Urine vitamin C: 2–10 mg/6 hr.
(10) Urine creatinine: 0.25–0.40 gm/6 hr.
(11) Urine N'Methylnicotinamide: 0.6–2.0 mg/6 hr.

2. Replication of Determinations

Because of the limited amount of blood that is drawn it is not possible to do serum determinations in duplicate. With urine, the method for thiamine is very time consuming and duplicate determinations are not possible. For urinary riboflavin and N'Methylnicotinamide, however, one investigator can do 20 to 30 samples, in duplicate daily. If the laboratory director desires, as an alternative every tenth sample may be run in duplicate. The creatinine method is sufficiently straightforward and duplicates are not necessary.

3. Sensitivity of Methods

It is to be expected that in sampling large populations some individual values will be found that fall below the limits of sensitivity of the methods employed. Although values may be calculated for such determinations, the analyst should understand at what point values lose significance because of limitations of the method.

a. Colorimetric Methods

(1) *Plasma or Serum Ascorbic Acid.*—Due to the very low readings (<0.015 density units) of samples containing less than 0.2 mg. percent ascorbic acid, values lower than this will have an appreciable error. Thus, when readings are in this range size of the sample, whenever possible, should be increased.

(2) *Plasma or Serum Vitamin A.*—Samples containing 15 mcg. percent will give an optical density reading of about 0.04. Values less than this should not be calculated but should be recorded as <15 mcg.

[1] Ranges are averages of values reported in the literature. National Academy of Sciences-National Research Council, Washington, D. C. Methods for Evaluation of Nutritional Adequacy and Status. December 1954.
Albritton, E. C., ed. Standard Values in Nutrition and Metabolism. Philadelphia, Saunders. 1954

(3) *Plasma or Serum Carotene.*—Samples containing 30 mcg. percent will give an optical density reading of about 0.03. Values less than this should not be calculated but should be recorded as <30 mcg.

b. Fluorometric Methods

In general, the errors encountered in fluorometry are greater than those in colorimetry. In the analysis of urine, considerable variation will occur from sample to sample with respect to interfering substances. Very concentrated urines (less than 200 ml./6 hrs.) will frequently give abnormally high readings and in such cases the sample should be diluted and then determined. When samples read out of range, either high or low, it is a simple matter to reset the instrument with the fluorescent standard (quinine or fluorescein).

II. Laboratory Personnel—Minimum Requirements

In addition to the biochemist member and two laboratory assistants of the survey team, the following personnel should be made available by the host country:

Minimum of three biochemists or chemists, preferably people who may be available to continue in biochemistry and nutrition research.

Minimum technicians—

Four laboratory technicians, pharmacists, or engineers.

Four laboratory helpers.

Interpreters—One, preferably two.

Secretary (bilingual)—able to type English and native tongue.

III. Collection and Storage of Samples

1. Planning and Preparation at the Field Laboratory

The biochemical tests are to be performed on both blood and fasting urine specimens from a sample of the men who are given clinical examination. The number of biochemical samples to be collected depends upon the number of men examined clinically, the satisfaction of statistical requirements for a sufficient sample size and the ability of the laboratory properly to handle (or store) a given number of samples. The standard analyses to be made are: hemoglobin, hematocrit, total plasma proteins, vitamin C, vitamin A and carotene on the blood; riboflavin, thiamine and N'Methylnicotinamide on the urine. The first three of these will generally be determined in the field laboratory. Certain other analyses may be required as the situation demands.

Once the men have entered the clinical detailed examination phase, the biochemical sample is systematically selected as every fifth man in the line. Lists are prepared in triplicate using name, serial number, McBee card number, and unit designation. One list is given to the commanding officer so that he can arrange for all the men to be quartered together on the night of the urine collection. One duplicate is used in checking off the men during the collection and a third is reserved for the field laboratory files. As the blood samples (clotted and unclotted) are drawn McBee item No. 100 is marked. Later as the urine is collected, item No. 101 is marked.

The collection of the blood and urine specimens should be made at a time during the field survey to assure that a minimum of time will elapse

between the collection and transportation of the samples to the main laboratory. To this end, the entire urinary sample from a locale might be taken at one time, near the end of the clinical survey. In certain cases it may be necessary to have some of the men give urine samples prior to their clinical examination.

The field laboratory equipment should be prepared and packed in the main laboratory in such a manner as to facilitate its use in the field. As nearly as possible the supplies and the method of packing should be standardized; the containers used for the transportation should be such that they can be used over again routinely, and of course, the containers must be convenient to handle.

In most cases it will be convenient to prepare all of the sterile syringes and needles needed in the main laboratory rather than attempt the sterilizations in the field. The used equipment may be returned clean but unsterile to the main laboratory; on arrival it should be recleaned, sterilized and repacked for the next field trip. The same is true of all of the vials to be used in the storage of whole blood, plasma, vitamin C filtrates, and urine aliquots. The anticoagulant is dried onto the centrifuge tubes and whole blood collection vials in the main laboratory; the vials and tubes equipped with blank labels and stoppers are then packed in their special containers. The vials intended for the preparation of the vitamin C filtrates should also be labeled and packed, but the metaphosphoric acid must be made up in the field and pipetted into the vials just prior to use; for this, it is most convenient to carry preweighed pellets of HPO_3 and add the requisite volume of redistilled deionized water in the field laboratory.

The field laboratory for the determination of total plasma proteins, hemoglobin and hematocrit should be located, if possible, in the detailed clinical examination area.

The most important feature of both the blood and urine collection is to have free movement of the samples to avoid confusion and duplication of responsibility. Try to anticipate the needs for water and electricity so as to avoid delays.

Each technician must clearly understand his responsibilities and must be taught to appreciate the virtues of teamwork. If the supervising technician can be free to move in where needed, much time can be saved and the free flow of samples can go on unimpeded.

It is absolutely essential to keep the blood and plasma in the cold at all times.

2. Anticoagulants

a. Oxalate Mixture (Heller and Paul)

Dissolve 6 gm. ammonium oxalate and 4 gm. potassium oxalate in 250 ml. of water. Pipette into pyrex test tubes, 125 x 16 mm, 0.25 ml. of the oxalate solution. (This amount of oxalate mixture is sufficient for 5 ml. of blood.) Spread the oxalate solution in a film over the lower walls of the test tube and dry in an incubator at not over 50° C.

Five ml. vacutainers when specified to contain Heller and Paul oxalate mixture will contain 10 mg. of oxalate per 5 ml. of whole blood.

b. Heparin

This anticoagulant is available either as the dry sodium salt, or as a solution containing 1,000 unit ml. (10 mg/ml.).

For the prevention of clotting, about 0.2 mg. heparin per ml. blood is sufficient. Prepare a solution of heparin in *distilled water,* containing 10 mg/ml.

For the field, pipette 0.3 ml. of this solution into a 15 ml. centrifuge tube. Place the tubes in an oven at 100° C. and evaporate off the water.

3. Fractions of Blood and Urine To Be Collected

The fractions of blood and urine to be collected and their intended uses are:

a. One to five ml. whole blood (collected in small vial containing anticoagulant; this sample need not be chilled) for determination of hemoglobin and hematocrit.

b. If the dye method for vitamin C is used, 8–10 ml. of whole clotted blood are chilled, stored at icebox temperature (*not* frozen) until ready to complete the analyses. In this case all blood analysis will be in terms of serum instead of plasma.

c. Plasma prepared from whole blood (collected in centrifuge tube containing anticoagulant).[1]

(1) For the determination of total plasma protein.

(2) For the preparation of the vitamin C stabilization filtrate if the 2, 4-dinitrophenylhydrazine method is used.

(3) Remainder of plasma for storage and transport to the main laboratory for the determination of: vitamin A and carotene and other analyses as required.

d. Urine (50 ml. aliquot of measured sample acidified with 0.1 ml. HCl).

(1) For storage and transport to the main laboratory for the determination of: thiamine, riboflavin, N'Methylnicotinamide and creatinine if desired.

(2) If required, a vitamin C filtrate is prepared.

[1] As soon as feasible the preparation of the plasma should begin. It is advisable to be prepared to determine the specific gravity of the plasma as soon as it is separated, and at the same time take the aliquot for vitamin C filtrate; this will avoid having to remove the plasma from the cold.

4. Equipment for Field Collection of Samples [1]

The following list of equipment to be taken into the field assumes that approximately 80 to 100 samples will be taken. It should fit conveniently into three or four boxes approximately 2½ feet by 3 feet by 2 feet.

a. Urine Sample

(1) Polyethylene bottles, 1 liter complete with labels and containing 100 mg. of oxalic acid (it is convenient to carry about 8 liters of distilled water in some of the bottles) ..... ea.—110

(2) Bottles, brown 2 oz., screw cap; with labels ..... ea.—110

(3) Cylinders, grad., one 100 ml., one 500 ml., and one 1 liter ..... ea.— 1

(4) Oxalic acid, CP, ¼ lb ..... ea.— 1

(5) Hydrochloric acid, conc. 50 ml ..... ea.— 1

(6) Enameled cups, 1 pint ..... ea.— 3

(7) Spatula, 100 mg ..... ea.— 1

(8) Serological pipette, 10 ml ..... ea.— 6

If vitamin C in urine is to be determined:

(9) Acid metaphosphoric CP, 40 gms. in 1 liter polyethylene bottle to be diluted to 1 liter in the field ..... ea.— 2

(10) Syringe pipette, 5 ml ..... ea.— 1

(11) Vials, 23 ml., screw cap, with label, to receive 8 ml. HPO_3 solution ..... ea.—110

[1] See Chapter 4, paragraph V, 1., for alternative procedure for drawing blood samples using the vacutainer technique. See paragraph V, 2 for methods which are acceptable substitutes for those listed in paragraphs III, and IV, 3.

b. Blood Sample

(1) Vials, screw cap, with labels:
9 ml. containing anticoagulant (for whole blood) ..... ea.—110
9 ml. (for storage of plasma) ..... ea.—110
23 ml. to receive 12 ml. HPO_3 solution (for vitamin C) ..... ea.—110

(2) Centrifuge tubes, 15 ml., with labels, corks and containing anticoagulant ..... ea.—160

(3) Syringes, 20 ml., packed with needles (20 gage, sterile) ..... ea.—130

(4) Needles, 20 gage (extra) ..... ea.— 30

(5) Gauze sponges (2″ x 2″), package of 100 ..... ea.— 3

(6) Basins, stainless steel, 8 quarts ..... ea.— 2

(7) Brushes, test tube ..... ea.— 2

(8) Detergent, 100 gm. bottle ..... ea.— 1

(9) Evaporating dish, 200 ml. (for alcohol swabs) ..... ea.— 3

(10) Alcohol 95 percent, 1 pint ..... ea.— 1

(11) Alcohol 70 percent, 1 pint ..... ea.— 3

(12) Hematocrit tubes and caps ..... ea.—110

(13) $CuSO_4$ specific gravity 1.018–1.033 for plasma protein and specific gravity 1.045–1.069 for hemoglobin ..... 100 ml. ea.

(14) Wooden applicators, box ..... ea.— 1

(15) Syringes, 1 ml ..... ea.— 12
Syringes, 5 ml ..... ea.— 4
Syringes, 10 ml ..... ea.— 6

(16) Racks (for whole blood vials) ..... ea.— 6

(17) Wire test tube racks, 40 hole ..... ea.— 6

(18) Wire basket 6″ x 6″ (partitioned to hold centrifuge tubes in insulated cans) ..... ea.— 2

(19) Needles, No. 15, 4 inch ..... ea.— 6
Needles, No. 22, 1 inch ..... ea.— 12
Needles, No. 22, 6 inch (for hematocrit tubes) ..... ea.— 4

(20) Syringe pipette, 5 ml ..... ea.— 1

(21) Pipette, serological, 10 ml ..... ea.— 6

(22) Insulated storage container, 4 gallon cap ..... ea.— 2

(23) Masking tape, 1-inch roll ..... ea.— 1

(24) Interval timer ..... ea.— 1

(25) Rubber tubing for tourniquet, 2 ft. lengths ..... ea.— 4

(26) Lantern, large, 2 spare batteries ..... ea.— 1

(27) Saline, physiological, 250 ml. bottle ..... ea.— 1

(28) Towels, laboratory ..... ea.— 10

(29) Clinical centrifuges (transformers if necessary) ..... ea.— 2

(30) Pencil sharpener ..... ea.— 1

(31) Lab coats ..... as needed

(32) Cleansing tissue, box ..... ea.— 1

(33) Pencils, No. 2 ..... ea.— 6

(34) Tablets, writing, 8″ x 11″ ..... ea.— 3

(35) Carbon paper, 8″ x 11″ sheet ..... ea.— 6

(36) Twine, ball ..... ea.— 1

(37) Pencil, red wax ..... ea.— 3

(38) Acid metaphosphoric acid CP, 60 gms. in 1 liter polyethylene bottle to be diluted to 1 liter in the field with deionized water .. ea.— 2

If the blood drawing equipment is to be sterilized in the field:

(39) Sterilizer (transformer if necessary) ..... ea.— 1

(40) Water deionizer equipment ..... ea.— 1

(41) Stove, gasoline, 2 burner ..... ea.— 1

(42) Tongs ..... ea.— 1

(43) Forceps ..... ea.— 2

5. Procedure for Blood Sampling [1]

a. Sterilization and Care of Needles and Syringes

Needles should be unplugged by using pressure from a syringe; use a stylet if necessary. Check each needle for burrs and sharpness. If the needles require sharpening, use an oiled (mineral oil) stone hone. Syringes should be checked for sharp edges or chips on the barrel. These are to be discarded. Wash needles and syringes in soapy water, rinse thoroughly in distilled water. Then sterilize by placing individual syringes, barrels and needles in cotton plugged tube or rolled in gauze, in either Autoclave or boil in water bath for a minimum of 20 minutes. Place syringe barrel and plunger side-by-side so as to facilitate assembly. If a drying oven is available, place sterilized needles and syringes in oven at 110° C. If oven is not available, store needles in alcohol 70–80 percent (by volume). Syringes should be handled with sterile tongs for assembling. Be sure to match numbers of barrel and plungers.

b. Drawing Blood [2]

(1) Needles and syringes are to be checked and sterilized as described above. This should be accomplished during the previous day.

(2) The 6-foot table is arranged so that the subject sits on a chair at one end with his right arm straightened over the table conveniently for one man to draw blood from an antecubital vein. Racks containing centrifuge tubes and vials with anticoagulant are placed near the subject's arm. Dishes containing sponges and needles in alcohol are placed conveniently within reach of the man drawing blood. At the other end of the table is placed a dishpan containing clean water. The space in the middle of the table is reserved for syringes.

(3) A crew of three men engages in these activities. The subjects are asked to line up single file. Each man comes up in turn, sits in the chair, and straightens out his arm over the table. One man cleans and sterilizes the arm area. The operator applies a tourniquet and draws the blood. The second man on the side opposite the chair labels a centrifuge tube and a vial with the subject's identification. When about 15 ml. of blood have been drawn, the needle is removed from the arm, a cotton gauze moistened with alcohol is placed in the antecubital space and the subject is released with instructions to maintain firm pressure by hand for 5 minutes. The mere flexing of the elbow in some instances is not enough to prevent hematomas.

(4) Remove the needle from the syringe.

(5) The man who has labeled the vial and centrifuge tube holds the tube and vial in such a position that the operator can easily

[1] See section V, 1., for vacutainer method.

[2] Based on Consolazio, Johnson, and Marek: Metabolic Methods, St. Louis, The C. V. Mosby Co., 1951.

introduce about 2 ml. of blood into the vial and the remainder of the blood into the centrifuge tube. *Do Not Shake.* He caps the vial and stoppers the tube, and mixes the blood in both vial and centrifuge tube thoroughly by inverting at least 20 times.

(6) The third member of the crew washes dirty syringes and needles immediately after receiving them from the operator. A sterile needle is fitted on a clean syringe and the operator prepares to draw blood from a new subject.

(7) Another member of the team begins centrifuging the blood when a sufficient number of samples are available.

c. Precautions in Drawing Blood

(1) The man washing syringes must be sure that needles do not become plugged; if so, unplug. Chipped syringes must be discarded.

(2) Sterilization of needles and syringes is mandatory so as to prevent the spread of infection, such as hepatitis.

(3) Be sure that oxalated or heparinized tubes are completely dry before use.

d. Determinations Requiring Whole Blood

Hemoglobin and hematocrit

e. Preparation of Plasma

(1) The tubes containing blood and anticoagulant which have been mixed are centrifuged for no more than 10 minutes at 3,000 r.p.m.

(2) Vials for plasma are labeled and numbered to correspond with the samples in the centrifuge. It has proved unsatisfactory to label many vials at once, owing to mix-ups in transferring samples of plasma when this is done.

(3) Plasma is removed with a 3½-inch No. 15 or 16 needle fitted to a 10 ml. syringe and transferred to corresponding numbered vial and capped. Before refrigerating the plasma, pipette aliquots for the determination of total plasma proteins and the preparation of the vitamin C filtrate.

(*a*) Preparation of plasma for vitamin C determination by the 2,4-dinitrophenylhydrazine method (Roe-Keuther revised method):

In an 18 ml. screw-cap vial containing 12 ml. 6 percent metaphosphoric acid solution add 4 ml. of plasma and mix well. Refrigerate immediately.

(*b*) Determine the specific gravity of the plasma as soon as it is removed from the centrifuge so as to avoid having to warm it up after refrigeration.

(4) Samples of plasma and whole blood (if it is to be transported to the main base laboratory) should be placed in the insulated cans with proper icing and packing. The plasma vials and vitamin C vials may be put into a plastic bag, tied securely and transported in the insulated cans surrounded by ice water.

(5) Precautions in preparing plasma:

(*a*) Blood and anticoagulant must be mixed *thoroughly,* but gently (do not shake).

(*b*) There must be adequate padding in the bottoms of the brass centrifuge cups and all centrifuge tubes must fit loosely in the cups without their lips touching the brass. If this is not done, there will be breakage.

(*c*) Hemolysis is best prevented by making sure that centrifuge tubes are dry, that the needle is removed from the syringe before blood is introduced into the centrifuge tube and that blood is *introduced gently down the side.*

6. Procedure for Urine Sampling

a. At the base laboratory the collection and storage bottles should be labeled and approximately 100 mg. of oxalic acid added by means of a calibrated small spatula to each collection bottle. The functions of the oxalic acid are: (a) to stabilize ascorbic acid; and (b) to bring the acidity of the specimen to pH 3 to 5, which is the range of maximum stability of thiamine, riboflavin and N'Methylnicotinamide.

b. Collect fasting specimens in the morning over a known period of time. Subjects are aroused at 12 midnight and requested to empty their bladders. Each man is given a numbered plastic bottle. They are then given a half-pint of water to drink so as to insure diuresis and return to their beds. The men should be briefed on the saving of urine in the collection bottles during this 6-hour fasting period. At 6 a.m. the men are aroused and asked to urinate (empty their bladders) into the plastic collection bottles.

c. The actual time of urination is recorded for each subject.

d. It is convenient to have a crew of three men for measuring and storing the urine. The first man measures the volume of each specimen, reports it to the recorder, and pours an aliquot of about 50 ml. into the 2-oz. bottle, pouring excess urine into a waste jar. The second man records the volumes in a notebook. The third member of the crew is responsible for putting identification numbers on the 2-oz. bottles, to correspond with the sample from the collection bottle. One-tenth ml. of conc. HCl is added to each storage bottle for preservation of vitamins and the tops are placed on the containers.[1]

e. The numbered specimens are placed in racks and stored in a cool (4°–10° C.) place.

f. Calculations.—For ease in calculation, all urinary excretion data will be expressed as the urinary excretion for 6 hours.

[1] Based on Consolazio et al., *loc. cit.*

g. Suggested plan for recording data in notebook:

Unit .....

Date .....

Time of urine collection .....

Laboratory working number	Name and number of subject	Army serial number	Six-hour urine volume	Time of collection

IV. Prescribed Biochemical Methods

1. Hemoglobin and Plasma Protein

(Copper sulfate specific gravity method)

REFERENCES [1]

Phillips, et al., J. Biol. Chem., 183: 305–330, March 1950.
Van Slyke, et al., J. Biol. Chem., 183: 331–347, March 1950.
Van Slyke, et al., J. Biol. Chem., 183: 349–360, March 1950.

a. Principle

Specific gravity measurements are made by observing the rise or fall of drops of whole blood, plasma or serum dropped into a graded series of copper sulfate solutions of known specific gravities. The relationship between the observed specific gravity of the specimen and the plasma or serum protein and the hemoglobin concentration may be calculated from empirically established formulas or read from charts or nomograms based on these formulas.

The equations expressing the relationships between specific gravity and protein concentrations vary depending upon the particular method

[1] We are indebted to the Journal of Biological Chemistry and the Authors for permission to cite the procedures, tables and charts herein used.

of estimating specific gravity and whether the blood or plasma is normal or abnormal in content of the protein. Furthermore, the effect of the encasement of the copper proteinate sac about plasma is such as to cause plasma drops to "balance" (neither rise nor fall) in a $CuSO_4$ solution that averages lighter than the actual specific gravity (as measured by a pycnometer) of the plasma by 0.0007 gravity units. In the method here described the compensation for the difference is made by labeling the specific gravity of the bottle in terms of the specific gravity of the plasma drop which it balances; e.g., a $CuSO_4$ solution labeled 1.0270 balances a plasma drop of specific gravity 1.0270, but in fact the solution has an actual gravity of 1.0270–0.0007 = 1.0263 (Phillips et al., J. Biol. Chem. 183, 305 (1950)). Drops of whole blood for estimation of hemoglobin, balance in $CuSO_4$ solutions that have on the average the same specific gravity as the blood.

The specific gravity standards for both hemoglobin and for plasma proteins are made to permit a variation due to dilution during use of ± 0.0001 gravity unit from the label values—e.g., the standards are initially 0.0001 unit greater than the label indicates and may be used until they are 0.0001 unit lighter than labeled.

No corrections are needed for heparinized samples if the amounts of heparin used are within the range of 0.1 to 0.2 mg. per ml. of blood.

For oxalated samples, using the 3:2 ammonium potassium oxalate in amounts of 1 mg. per ml. of blood, subtract 0.0004 from the observed G_B and observed G_p. Failure to do so will result in an overestimation of plasma protein by 0.15 gm. per 100 ml., and of hemoglobin by varying amounts up to 0.1 gm. per 100 ml.

b. Apparatus

(1) Forty-one 4 oz. bottles of copper sulfate solution from sp. gr. 1.018 through 1.033 (for plasma) and 1.045 through 1.060 (for whole blood) at intervals of 0.001 unit. These bottles should have the specific gravity marked clearly on them.

(2) Tuberculin syringes—1 ml.

(3) Hypodermic needles—No. 22 or finer.

(4) Stylets for cleaning needles.

(5) Vials containing anticoagulant (as listed under section for collecting blood samples).

(6) Specific gravity hydrometers (1.000–1.070 and 1.060–1.130)—150 mm.

(7) Volumetric flasks—1 liter.

(8) Thermometer—10° to 110° C.

(9) Funnel, glass—110 mm.

(10) Burette—50 ml.

(11) Cotton, gallon jug and wooden applicators.

c. Reagents

(1) Copper sulfate, solid, "analytical reagent" $CuSO_4 \cdot 5H_2O$ finely granular or powdered. Use sealed bottles of analyzed purity.

(*a*) Copper sulfate sp. gr. 1.100. The preferable method is to dissolve exactly 170 gm. of pure $CuSO_4 \cdot 5H_2O$ in amounts of water listed below for the recorded temperature of the solution. Use sealed bottles of "analytical

reagent" grade or of analyzed purity. It is convenient to use previously prepared bottles containing 170 ± 0.1 gm. of "fine crystals" of appropriate purity.

Temperature °C.	*ml of Water*
10–17°C.	1004
18–22°C.	1005
23–26°C.	1006
27–29°C.	1007
30–33°C.	1008
34–36°C.	1009
37–38°C.	1010
39–40°C.	1011

(*b*) An alternate method for preparation of copper sulfate solution, sp. gr. 1.100 applicable if balances are not available or previously weighed $CuSO_4$ cannot be obtained, is as follows:

Four pounds of copper sulfate are placed in a gallon jug. About 2.5 liters distilled or rain water are added. (Tap water may be used if it is not more than sp. gr. 1.0003 compared with distilled water as 1.0000 at the same temperature. Use hydrometer for this comparison.) The bottle is stoppered and shaken vigorously for 5 minutes at the end of which time the temperature is recorded to the nearest 0.5° C. The saturated supernatant solution is *immediately* decanted and is filtered through cotton into a clean dry jug. The solution of sp. gr. 1.100 is now made at once by diluting accurately to 1 liter the amount of saturated solution indicated in table 4. Once made, the solution of sp. gr. 1.100 keeps indefinitely. Prepare 8 liters at a time.

(2) Copper sulfate solutions. These solutions are most conveniently made according to table 5 by running the stock solution of sp. gr. 1.100 from a burette into a 100 ml volumetric flask, diluting to the mark with water, mixing, adding to the appropriate bottle, rinsing the flask, and starting over. These solutions should be changed when 100 drops of whole blood or plasma or serum have been added. It is therefore advisable to make up in the laboratory about 500 ml. each of those most commonly used.

d. Procedure

(1) The amounts needed are about 0.5 ml. each of whole blood and plasma prepared as described in a previous section. The whole blood must be mixed thoroughly by inversion of its container immediately before use. The samples and solutions of copper sulfate must all be at approximately the same temperature. (Avoid the use of cold solutions.)

(2) The range of specific gravity for hemoglobin determination at 0.001 intervals is from 1.045 through 1.069 and for plasma protein the range is 1.018 through 1.033 at 0.001 intervals. Fill a one ml. syringe, with its needle attached, to about the 0.2 ml. mark. If the syringe has just been used for a pre-

ceding estimation, empty it so far as practicable by pulling the plunger back and forth several times before finally filling the syringe with the new sample.

(3) With the copper sulfate bottles in a convenient position, remove the stopper from specific gravity 1.060 for hemoglobin and 1.028 for plasma protein. Hold the syringe in an almost vertical position with the tip of the needle about one-half inch above the solution.

(4) Deliver a drop from the syringe keeping steady pressure on the plunger. For satisfactory estimation the drop must break the surface and fall at least an inch into the solution without leaving a streamer attached to the surface.

(5) Watch the progress of the drop in the solution. When its specific gravity is exactly the same as that of the solution, the drop will fall for a few seconds, come to a complete halt without moving up or down for about 10 seconds, and will then slowly fall to the bottom because it will take up copper and become heavy. When its specific gravity is greater than that of the solution, the drop will continue to fall to the bottom. If the drop is lighter than the solution, it will fall for a few seconds, halt for a second or two, and begin to rise. It will continue to rise until it takes up enough copper to fall to the bottom. If a drop rises in one bottle and falls in the next lightest, the specific gravity lies between those of the two bottles, and must be estimated to the nearest 0.0005 by noting carefully the relative rates of rise in one bottle and fall in the next.

(6) Record the specific gravity to the nearest 0.0005 and proceed to the next sample.

e. Precautions

(1) When particles remain on the surface or when the drops fail to break the surface cleanly, the surface should be cleaned with a wooden applicator before the next drop is added.

(2) The ranges of specific gravity suggested above are for normal soldiers, in whom hypoproteinemia and anemia are extremely rare. For other populations, other ranges might be necessary.

3) Use fresh $CuSO_4$ solutions (from the reserves originally prepared) after every 100 to 120 samples. Change *all* bottles, not just the ones most commonly used.

(4) Avoid bubbles, even minute ones, in the blood drops.

(5) Temperature may vary from 4–40° C. without significantly affecting results so long as the $CuSO_4$ solutions and the blood samples are within 5° of the same temperature. If cold standards are brought into a warm room, let stand to within 1°–2° of room temperature and then shake vigorously prior to using. Do not leave solutions near a stove, on a window sill, or in other locations which may result in convection currents in the solutions. Keep covered when not in use.

(6) When standards are carried from low to high altitudes, it is essential that they be shaken before using in order to discharge the dissolved air with which they are supersaturated.

(7) Do not apply to blood or plasma which has been stored in the icebox for more than 18 hours.

f. Calculations

For normal plasmas obtained from heparinized blood the relationship between specific gravity measured by this method and Kjeldahl determined total plasma proteins is:

$p = 373\ (G_p - 1.0070)$ where p = Total plasma protein in gm. per 100 ml.
G_p = Observed gravity of the plasma

For pathological plasmas the relationship is:

$p = 365\ (G_p - 1.0070)$.

The nomogram (page 65) is based on these two relationships using, however, the averaged constant 369 to give the formula $p = 369\ (G_p - 1.0070)$.

The relationship between whole blood specific gravity and hemoglobin concentration must take into account the contribution of the plasma proteins to the specific gravity. The relationship is expressed as follows:

$$Hb_B = 33.9 \times \frac{G_B - G_p}{1.0964 - G_p}$$

where Hb_B = Blood hemoglobin in gm. per 100 ml.
G_B = Specific gravity of whole blood
G_p = Specific gravity of plasma

The use of the line chart for calculating plasma protein and hemoglobin for heparinized samples avoids the necessity of making individual calculations.

If oxalated blood or plasma is used the correction for the contribution of the oxalate to the specific gravity must be applied (see page 66). This correction consists of subtracting 0.0004 from the observed G_B and G_p. The charts have been calculated with recognition of this correction for oxalate so that the observed specific gravity of the oxalated sample may be used directly to convert results. This correction applies only when the ammonium oxalate-potassium oxalate mixture is used. Should serum be employed instead of plasma the observed specific gravity should be corrected by the addition of 0.0005 specific gravity units. The specific gravity of whole blood without anticoagulant need not be corrected. In this instance the conversion must be carried out on the nomogram.

(1) Hemoglobin:

(*a*) Heparinized samples:

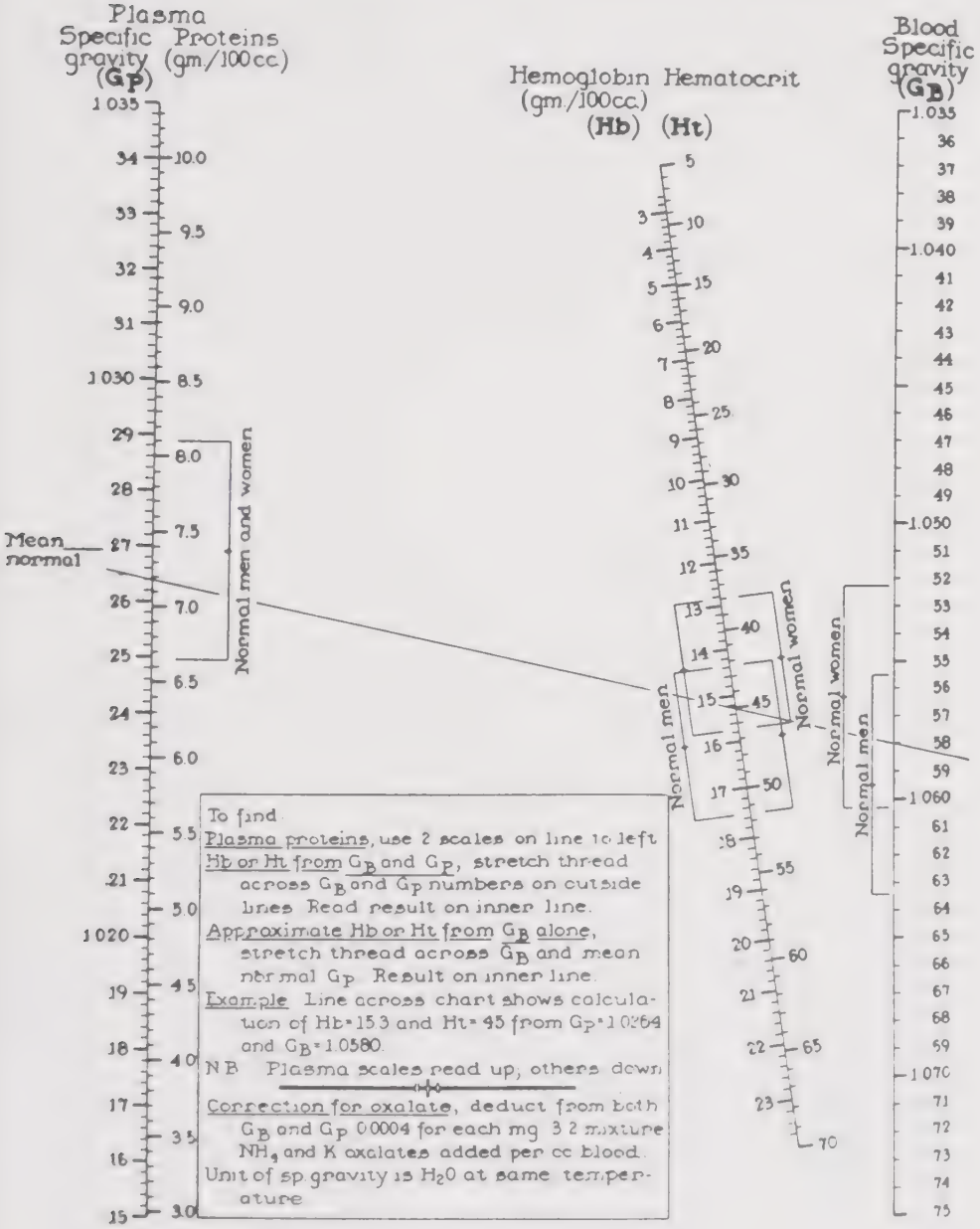
These values were calculated using the following equation:

$$Hgb = 33.9\ \frac{G_B - G_p}{1.0964 - G_p}$$

This equation was obtained from the reports of Van Slyke, et al., J. Biol. Chem., 183: 349–360, 1950

HEMOGLOBIN FROM BLOOD SPECIFIC GRAVITIES

Line chart for calculating plasma proteins, hemoglobin and hematocrit from gravities of plasma and blood



G_B ↓ / G_p →	1.018	1.019	1.020	1.021	1.022	1.023	1.024	1.025	1.026	1.027	1.028	1.029	1.030
	Gm. Hb/100 ml.												
1.045	11.67	11.39	11.09	10.79	10.48	10.16	9.83	9.50	9.15	8.79	8.43	8.05	7.66
1.046	12.11	11.83	11.54	11.24	10.94	10.62	10.30	9.97	9.63	9.28	8.92	8.55	8.17
1.047	12.54	12.26	11.98	11.69	11.39	11.08	10.77	10.45	10.11	9.77	9.42	9.05	8.68
1.048	12.97	12.70	12.42	12.14	11.85	11.55	11.24	10.92	10.59	10.26	9.91	9.56	9.19
1.049	13.40	13.14	12.87	12.59	12.30	12.01	11.71	11.39	11.08	10.75	10.41	10.06	9.70
1.050	13.84	13.58	13.31	13.04	12.76	12.47	12.17	11.87	11.56	11.23	10.90	10.56	10.21
1.051	14.27	14.02	13.76	13.49	13.21	12.93	12.64	12.34	12.04	11.72	11.40	11.07	10.72
1.052	14.70	14.45	14.20	13.94	13.67	13.39	13.11	12.82	12.52	12.21	11.89	11.57	11.23
1.053	15.13	14.89	14.64	14.39	14.13	13.86	13.58	13.29	13.00	12.70	12.39	12.07	11.74
1.054	15.57	15.33	15.09	14.84	14.58	14.32	14.05	13.77	13.48	13.19	12.89	12.57	12.25
1.055	16.00	15.77	15.53	15.29	15.04	14.78	14.52	14.24	13.96	13.68	13.38	13.08	12.76
1.056	16.43	16.21	15.97	15.74	15.49	15.24	14.98	14.72	14.45	14.17	13.88	13.58	13.27
1.057	16.86	16.64	16.42	16.19	15.95	15.70	15.45	15.19	14.93	14.65	14.37	14.08	13.78
1.058	17.30	17.08	16.86	16.64	16.40	16.16	15.92	15.67	15.41	15.14	14.87	14.59	14.30
1.059	17.73	17.52	17.30	17.08	16.86	16.63	16.39	16.14	15.89	15.63	15.36	15.09	14.81
1.060	18.16	17.96	17.75	17.53	17.31	17.09	16.86	16.62	16.37	16.12	15.86	15.59	15.32

FACTORS FOR COMPUTATION

X	432. 3980	437. 9845	443. 7173	449. 6021	455. 6452	461. 8529	468. 2320	474. 7899	481. 5341	488. 4726	495. 6140	502. 9674	510. 5422

$$33.9 \frac{G_B - G_p}{1.0964 - G_p} = X(G_B - G_p)$$

(*b*) Oxalated sample:

For use with samples containing 2 mg./ml. of oxalate mixture. These values were calculated using the following equation:

$$Hgb = 33.9 \frac{G_B - G_p}{1.0972 - G_p}$$

This equation was obtained from the reports of Van Slyke, et al. (J. Biol. Chem., 183: 305–360, 1950) and corrected for the oxalate content noted above.

G_B ↓ / G_p →	1.018	1.019	1.020	1.021	1.022	1.023	1.024	1.025	1.026	1.027	1.028	1.029	1.030
	Gm. Hb/100 ml.												
1.045	11.56	11.27	10.98	10.68	10.37	10.05	9.73	9.39	9.05	8.69	8.33	7.95	7.57
1.046	11.98	11.70	11.42	11.12	10.82	10.51	10.19	9.86	9.52	9.18	8.82	8.45	8.07
1.047	12.41	12.14	11.86	11.57	11.27	10.96	10.65	10.33	10.00	9.66	9.31	8.95	8.58
1.048	12.84	12.57	12.30	12.01	11.72	11.42	11.11	10.80	10.47	10.14	9.80	9.44	9.08
1.049	13.27	13.01	12.73	12.46	12.17	11.88	11.58	11.27	10.95	10.62	10.29	9.94	9.58
1.050	13.70	13.44	13.17	12.90	12.62	12.34	12.04	11.74	11.43	11.11	10.78	10.44	10.09
1.051	14.12	13.87	13.61	13.35	13.07	12.79	12.50	12.21	11.90	11.59	11.27	10.94	10.59
1.052	14.55	14.31	14.05	13.79	13.52	13.25	12.97	12.68	12.38	12.07	11.76	11.43	11.10
1.053	14.98	14.74	14.49	14.24	13.97	13.71	13.43	13.15	12.86	12.56	12.25	11.93	11.60
1.054	15.41	15.17	14.93	14.68	14.43	14.16	13.89	13.62	13.33	13.04	12.74	12.43	12.11
1.055	15.84	15.61	15.37	15.13	14.88	14.62	14.36	14.09	13.81	13.52	13.23	12.92	12.61
1.056	16.27	16.04	15.81	15.57	15.33	15.08	14.82	14.56	14.28	14.00	13.72	13.42	13.12
1.057	16.69	16.47	16.25	16.02	15.78	15.53	15.28	15.02	14.76	14.49	14.21	13.92	13.62
1.058	17.12	16.91	16.69	16.46	16.23	15.99	15.75	15.49	15.24	14.97	14.70	14.41	14.13
1.059	17.55	17.34	17.13	16.91	16.68	16.45	16.21	15.96	15.71	15.45	15.19	14.91	14.63
1.060	17.98	17.77	17.56	17.35	17.13	16.90	16.67	16.43	16.19	15.94	15.68	15.41	15.14

FACTORS FOR COMPUTATION

X	428. 0303	433. 5038	439. 1192	444. 8819	450. 7979	456. 8733	463. 1148	469. 5291	476. 1236	482. 9060	489. 8844	497. 9674	504. 4643

$$33.9 \frac{G_B - G_p}{1.0972 - G_p} = X(G_B - G_p)$$

(2) Plasma Protein:

PLASMA PROTEIN (gm./100 ml.)
(2 mg./ml. Ammonium-Potassium Oxalate Mixture)

G_p	$P = 369(G_p - 1.0078)$	G_p	$P = 369(G_p - 1.0078)$
1.018	3.76	1.0245	6.16
1.0185	3.95	1.025	6.35
1.019	4.13	1.0255	6.53
1.0195	4.32	1.026	6.72
1.020	4.50	1.0265	6.90
1.0205	4.69	1.027	7.08
1.021	4.87	1.0275	7.27
1.0215	5.06	1.028	7.45
1.022	5.24	1.0285	7.64
1.0225	5.42	1.029	7.82
1.023	5.61	1.0295	8.01
1.0235	5.79	1.030	8.19
1.024	5.98	1.0305	8.38

PLASMA PROTEIN (gm./100 ml.)
(Heparinized Sample)

G_p	$P = 369(G_p - 1.0070)$	G_p	$P = 369(G_p - 1.0070)$
1.018	4.06	1.0245	6.46
1.0185	4.24	1.025	6.64
1.019	4.43	1.0255	6.83
1.0195	4.61	1.026	7.01
1.020	4.80	1.0265	7.20
1.0205	4.98	1.027	7.38
1.021	5.17	1.0275	7.56
1.0215	5.35	1.028	7.75
1.022	5.54	1.0285	7.93
1.0225	5.72	1.029	8.12
1.023	5.90	1.0295	8.30
1.0235	6.09	1.030	8.49
1.024	6.27	1.0305	8.67

TABLE 4.—Ml. of saturated copper sulfate solution to be diluted to 1 liter to make stock solution of specific gravity 1.1000 [1]

Temperature in °C. or °F. refers to the temperature of the saturated solution at the time of saturation (end of shaking for five minutes).

Temperature			Temperature			Temperature		
°C.	°F.	ml	°C.	°F.	ml	°C.	°F.	ml
10.0	50.0	578	20.0	68.0	488	30.0	86.0	425
10.5	50.9	573	20.5	68.9	484	30.5	86.9	423
11.0	51.8	568	21.0	69.8	480	31.0	87.8	420
11.5	52.7	563	21.5	70.7	477	31.5	88.7	417
12.0	53.6	558	22.0	71.6	473	32.0	89.6	414
12.5	54.5	553	22.5	72.5	469	32.5	90.5	412
13.0	55.4	548	23.0	73.4	466	33.0	91.4	409
13.5	56.3	543	23.5	74.3	463	33.5	92.3	406
14.0	57.2	539	24.0	75.2	460	34.0	93.2	403
14.5	58.1	534	24.5	76.1	456	34.5	94.1	401
15.0	59.0	529	25.0	77.0	453	35.0	95.0	398
15.5	59.9	525	25.5	77.9	450	35.5	95.9	395
16.0	60.8	521	26.0	78.8	447	36.0	96.8	392
16.5	61.7	516	26.5	79.7	445	36.5	97.7	390
17.0	62.6	512	27.0	80.6	442	37.0	98.6	387
17.5	63.5	508	27.5	81.5	439	37.5	99.5	384
18.0	64.4	504	28.0	82.4	436	38.0	100.4	381
18.5	65.3	500	28.5	83.3	434	38.5	101.3	379
19.0	66.2	496	29.0	84.2	431	39.0	102.2	376
19.5	67.1	492	29.5	85.1	428	39.5	103.1	373

[1] From Consolazio, et al., *loc cit.*

TABLE 5.—Volumes of stock copper sulfate solution specific gravity of 1.1000 to be diluted to 100 ml. and 500 ml. to prepare standard solutions for plasma and blood

Standard solutions for plasma			Standard solutions for whole blood		
Specific gravity	Volume (ml.) of stock $CuSO_4$ solution per standard		Specific gravity	Volume (ml.) of stock $CuSO_4$ solution per standard	
	100 ml.	500 ml.		100 ml.	500 ml.
1.015	13.9	69.5	1.045	44.2	221.0
1.016	14.9	74.5	1.046	45.2	226.0
1.017	15.85	79.25	1.047	46.2	231.0
1.018	16.8	84.0	1.048	47.2	236.0
1.019	17.8	89.0	1.049	48.2	241.0
1.020	18.8	94.0	1.050	49.2	246.0
1.021	19.8	99.0	1.051	50.2	251.0
1.022	20.75	103.75	1.052	51.25	256.25
1.023	21.7	108.5	1.053	52.25	261.25
1.024	22.7	113.5	1.054	53.3	266.5
1.025	23.7	118.6	1.055	54.3	271.5
1.026	24.7	123.5	1.056	55.3	276.5
1.027	25.7	128.5	1.057	56.3	281.5
1.028	26.65	133.25	1.058	57.3	286.5
1.029	27.6	138.0	1.059	58.3	291.5
1.030	28.6	143.0	1.060	59.3	296.5
1.031	29.6	148.0	1.061	60.3	301.5
1.032	30.6	153.0	1.062	61.3	306.5
1.033	31.6	158.0	1.063	62.3	311.5
			1.064	63.35	316.75
			1.065	64.4	322.0
			1.066	65.4	327.0
			1.067	66.4	332.0
			1.068	67.4	337.0
			1.069	68.4	342.0
Total volume	431.75	2,158.75	.....	1,407.25	7,036.25

Total volume of 9,195.0 ml. of stock $CuSO_4$ solution is required for 500 ml. of standard at each specific gravity level. For 100 ml. standard at each specific gravity level, total volume required is 1839.0 ml. of stock solution.

2. HEMATOCRIT

REFERENCES

Wintrobe, M. M., J. Med. Sc., 185: 58, 1933.

Gradwohl, R. B. H., Clinical Laboratory Methods and Diagnosis, St. Louis, 1935.

Kolmer, J. A., Approved Laboratory Technic, 3rd edition, New York and London, 1941.

Osgood, E. E., and Haskins, H. D., Laboratory Diagnosis, Philadelphia, 1940.

Todd, J. C., and Sanford, A. H., Clinical Diagnosis by Laboratory Methods, 12th edition, Philadelphia and London, 1953.

a. Principle

A sample of the blood is introduced into the Wintrobe hematocrit tube to the 10 mark (not above). It is centrifuged at 3,000 r.p.m. until no further packing of the cells occurs. The red cell volume percent is read directly on the scale on the tube from the height of the column of red cells.

b. Apparatus

(1) Centrifuge.

(2) Hematocrit tubes—Wintrobe with rubber caps.

(3) Transfer needles, 5-inch, No. 18.

(4) Two ml. syringe.

c. Procedure

Venous blood is drawn and prevented from coagulating, using the *mixed* oxalate. It is important to introduce the correct amount of blood into the ovalated vial (5 ml. of blood for the oxalated vial prepared according to directions on pages 4–6. After the sample has been *well mixed*, about 1 ml. is drawn into a syringe fitted with a transfer needle and the tip is passed to the bottom of the hematocrit tube and the blood is slowly expelled to fill the tube to the 10 mark. There should be no air bubbles. The tube is capped and centrifuged [1] at 3,000 r.p.m. (checked at intervals with Tachometer) for 30 minutes and the heights of the red cell column and of the plasma are read from the graduations on the tube. The reading should be made of the height of the red cells only and should not include the "buffy coat" above them. Centrifugation is continued for another 15 minutes in order to be sure that complete packing of the cells has been obtained.

d. Precaution

It is important that the centrifugal force, and not necessarily the r.p.m., be uniform from one run to the next. This is dependent upon the radius of the centrifugal head and the speed.

e. Calculation

$$\frac{\text{Reading of packed red cells}}{\text{Reading of plasma}} \times 100 = \text{percent red cell volume.}$$

3. Plasma or Serum Vitamin C

(Procedure for determining vitamin C in foods and urine are included as *elective procedures*). Two satisfactory methods for determining vitamin C in plasma are available—the modified Roe-Keuther procedure detailed below and the dye method using 2,6-dichlorobenzenone indophenol (sodium salt) (p. 83). The former method measures the total ascorbic acid, the latter the reduced vitamin. In applying the Roe-Keuther method, the sample should be stabilized as precipitated plasma as soon after taking as is feasible; for the dye method the vitamin C is most stable upon storage in clotted, unseparated blood at refrigerated temperature.

REFERENCES

Roe, J. H., and Kuether, C. A., J. Biol. Chem., 147: 399–407, 1943.
The Association of Vitamin Chemists, Inc., Methods of Vitamin Assay, Interscience Publishers, Inc., New York, N. Y., Chapter 7, p. 159, 1947.
Schaffert, G. R., and Kingsley, G. F., J. Biol. Chem., 212: 59–68, 1955.

a. Principle

Ascorbic acid is oxidized to dehydroascorbic acid in the presence of norite. The 2,4-dinitrophenylhydrazine derivative is treated with strong sulfuric acid producing a reddish colored product which is measured photometrically.

[1] Optimal packing is obtained by centrifugal force of 2260 × gravity (G). This relative centrifugal force depends upon the radius (r) at which a particle is distant from the center of revolution as well as upon the number of revolutions of the centrifuge per minute (r.p.m.). This relationship is expressed as follows: relative centrifugal force = $0.00001118 \times r \times \text{r.p.m.}^2$ The relationship between the radius and r.p.m. necessary to give the requisite centrifugal force is r.p.m. = $\sqrt{\frac{202,146,700}{r}}$.
Prolongation of time of centrifugation does not make up for insufficient centrifugal force.

b. Apparatus

(1) Coleman Jr. Spectrophotometer, Model 6, complete.
(2) Cuvettes, 19 x 150 mm.
(3) Centrifuge tubes, capacity of 50 ml.
(4) A boiling water bath.
(5) A quantity of 50 ml. Erlenmeyer flasks.
(6) An electric centrifuge.
(7) Quantitative filter paper, Whatman 42.
(8) An ice bath.
(9) Syringe pipettes 1 and 12 ml.
(10) Pipette transfer 4 ml.
(11) Pipette serological 5 and 10 ml.
(12) Interval timer.
(13) Funnels 65 mm.

c. Reagents

(1) 2,4-dinitrophenylhydrazine reagent: dissolve 2 gm. of the reagent in 100 ml. of 9 N sulfuric acid (3 parts of water and one part of concentrated sulfuric acid). Allow to stand overnight and filter.

(2) Acid-washed Norite (Charcoal): place 200 gm. Norite in a large flask. Add 1,000 ml. of 10 percent HCl and heat to boiling. Filter with suction. Remove Norite cake to a large beaker and add 1 liter distilled water. Stir thoroughly and filter. Repeat until washings give a negative or very faint test for ferric ion. (Test filtrate with 1 percent potassium ferrocyanide). Dry Norite cake in oven overnight at 110°–120° C.[1]

(3) Metaphosphoric acid, 6 percent solution: dissolve 60 gm. HPO_3 and dilute to 1 liter with redistilled water. Store in the cold.

(4) Metaphosphoric acid, 4 percent—(only for urine vitamin C): dissolve 40 gm. HPO_3 and dilute to 1 liter with redistilled water. Store in the cold.

(5) Sulfuric acid, 85 percent solution: to 100 ml. of distilled water add 900 ml. of concentrated sulfuric acid (sp. gr. 1.84). Do this mixing very carefully in a sink.

(6) Thiourea, 10 percent solution: dissolve 10 gm. thiourea in 100 ml. of 50 percent (by volume) aqueous ethyl alcohol. This reagent keeps satisfactorily for 2 months, but to check, see that it readily reduces $HgCl_2$ or $KMnO_4$.

(7) Standard vitamin C (USP reference standard if available): Stock standard—dissolve exactly 100 mg. l-ascorbic acid in 100 ml., 4 percent HPO_3 solution (1 mg./ml.).

(8) Working Standard: dilute 2 ml. of stock standard to 100 ml. with 4 percent HPO_3 solution adding 1 ml. of thiourea solution prior to diluting to volume (20 mcg./ml.).

(9) Standard curve: in a 50 ml. Erlenmeyer flask add 25 ml. of working standard l-ascorbic acid (20 mcg./ml.) and one-half teaspoon of Norite. Shake for 1 minute and filter. Set up a

[1] Based on **Consolazio** et al., *op. cit.*

standard curve using 0 to 3.0 ml. of this filtrate at 0.5 ml. intervals and dilute each to 4 ml. with 4 percent HPO_3 solution (each 0.5 ml. contains 10 mcg.). Continue as under par. d(4), "Development of Color."

d. Procedure

(1) *Plasma or serum*

(*a*) In a 50 ml. centrifuge tube add 12 ml. of the 6 percent HPO_3 solution.

(*b*) Now add 4 ml. of plasma dropwise with continuous mixing. (See precautions.)

(*c*) Allow to stand for 5 minutes and centrifuge for 10 minutes at 2,500 r.p.m.

(*d*) Pour the supernatant into a clean dry test tube, add one-half teaspoon of Norite and shake vigorously for 1 minute.

(*e*) Filter through Whatman No. 42 filter paper.

(2) *Urine* (Elective Procedure)

(*a*) In a 50 ml. centrifuge tube add 18 ml. of 4 percent HPO_3 solution.

(*b*) Add 2 ml. of urine and then add one-half teaspoon of Norite.

(*c*) Shake vigorously for 1 minute and filter.

(3) *Fruits and Vegetables* (Elective Procedure)

(*a*) In a 100 ml. volumetric flask add 2 gms. of food and dilute to volume with 0.5 percent oxalic acid.

(*b*) Mix in a blender for the minimum time necessary.

(*c*) To 20 ml. of the homogenate in a 50 ml. centrifuge tube add one-half teaspoon of Norite, shake vigorously for 1 minute and filter through Whatman No. 42 filter paper.

(4) *Development of Color*

(*a*) In each of two 19 x 150 mm. cuvettes add exactly 4 ml. of the filtrate (plasma, urine or food).

(*b*) To each, add 1 drop of 10 percent thiourea solution.

(*c*) Reserve one tube as a blank.

(*d*) To the other tube add 1 ml. of 2,4-dinitrophenylhydrazine solution, mix.

(*e*) Place both tubes in a boiling water bath for exactly 5 minutes for plasma and 5 and 10 minutes for urine and food, respectively.

(*f*) At the end of time, place the tubes immediately in a beaker containing crushed ice.

(*g*) Now add slowly, *drop by drop,* 5 ml. of 85 percent sulfuric acid (during a 1–1½ minute period) to both tubes with vigorous mixing. Keep an abundant supply of crushed ice in the beaker.

(*h*) To the blank tube add 1 ml. of the 2,4-dinitrophenylhydrazine solution.

(*i*) Remove the tubes from the ice bath and allow to stand at room temperature for 10 minutes.

(*j*) Set the instrument at 100 percent T at 515 mμ with a tube of distilled water and read the unknown and blank.

(*k*) Subtract the density of the blank from that of the unknown, and calculate, using the standard curve; it is advisable to include a working standard in the range of values expected each time the method is run.

(5) *Determination of Dehydroascorbic Acid.*—Dehydroascorbic acid is measured by the above procedure with the omission of the Norite.

e. Calculations

mg. vitamin C per 100 ml. plasma =

$$\frac{\text{microgram in cuvette} \times \text{total dilution} \times 100}{1000 \times \text{ml. aliquot} \times \text{ml. plasma}}$$

Example:

A reading of 0.110 Optical Density was equivalent to 10 mcg. of ascorbic acid in the cuvette. 4 ml. of plasma were diluted to 16 ml. of which 4 ml. were taken for analysis.

$$\text{mg. total ascorbic acid/100 ml. plasma} = 10 \times \frac{16}{4} \times \frac{1}{1000} \times \frac{100}{4} = 1.0 \text{ mg./100 ml.}$$

Total ascorbic acid = reduced ascorbic acid + dehydro-ascorbic acid.

f. Precautions

Under field conditions when there may be considerable delay in returning the samples to the main laboratory, it is convenient to precipitate the plasma proteins and stabilize the vitamin C by pipetting 4 ml. of plasma into 12 ml. of 6 percent HPO_3 contained in a 23 ml. vial and returning the sample to the laboratory in a refrigerator can. In certain cases with limited plasma, it may be necessary to use 2 or 3 ml. of plasma and then dilute to a final volume of 16 ml. to assure sufficient filtrate. Urine samples likewise should be stabilized.

4. Plasma or Serum Vitamin A and Carotene (Carr-Price)

REFERENCES

Consolazio, C. F., Johnson, R. E., and Marek, E., Metabolic Methods, The C. V. Mosby Co., 1951.
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a. Principle

The proteins of the plasma or serum are precipitated with alcohol, and the carotene and vitamin A are extracted with petroleum ether. The carotene concentration is determined by measuring the absorption of the extract at 450 mμ.

The petroleum ether is evaporated under nitrogen, and vitamin A is determined by reading the intensity of the blue color produced by the addition of antimony trichloride in chloroform. A correction is made

for the amount of carotene present, since carotene contributes to the total color.

b. Apparatus

(1) Special, rapid delivery, 1.0 ml. pipettes.
(2) Cuvettes—Coleman, 10 x 75 mm., and adapter.
(3) Glass-stoppered test tubes 16 x 150 mm. or 14 x 125 mm.
(4) An electric centrifuge.
(5) Hot plate.
(6) Tank of nitrogen.
(7) Water bath, 40–50° C. constant temperature.
(8) Pipette, 0.1, 2.0 and 3.0 ml.

c. Reagents

(1) 95 percent ethyl alcohol.
(2) Petroleum ether (20–40° C.).
(3) Chloroform, C.P.
(4) 20 percent antimony trichloride in chloroform (Dissolve one-quarter pound (113.4 gm.) $SbCl_3$ in 455 ml. chloroform. Filter through fluted funnel into brown bottle. Add Na_2SO_4 to cover bottom. Do not expose to room air for any length of time or it will pick up moisture.)
(5) Acetic anhydride.
(6) Anhydrous sodium sulfate, C.P., granular.
(7) Sodium potassium tartrate, C.P.

d. Procedure (On single samples, only)

(1) Place 2.0 ml. plasma or serum in a 16 x 150 mm. glass-stoppered test tube.
(2) Add 2.0 ml. 95 percent alcohol; mix.
(3) Add 3.0 ml. petroleum ether; shake vigorously for 2 minutes.
(4) Centrifuge slowly for 3 minutes.
(5) Carefully pipette off 2.0 ml. of the petroleum ether, use a 4 or 5 inch needle of as small a diameter as possible (No. 20 or 18), on a syringe pipette, and place in a 10 x 75 mm. cuvette.
(6) Read carotene at 450 mμ. Use a blank of petroleum ether.
(7) Evaporate to dryness in 40–50° water bath, under a stream of nitrogen.
(8) Take up the residue immediately in 0.1 ml. chloroform; add 1 drop of acetic anhydride from a No. 25 needle; cork the tube to prevent evaporation.
(9) Set the Coleman Jr. at 620 mμ, at zero optical density with a blank tube containing 0.1 ml. chloroform and 1.0 ml. $SbCl_3$ reagent. Remove the blank tube and place sample tube in the instrument. Add 1.0 ml. $SbCl_3$ reagent from a rapid-delivery pipette; and record the density reading at the pause point (3–5 seconds after the addition of the reagent). Immediately after noting the density reading, remove the tube and observe the color. Solution must be clear, and a blue color detectable.

e. Calculations

(1) Carotene

D450 $\times$ 1092 = Micrograms % carotene.

(2) Vitamin A

(D620 — (D450 × .248)) × 361 = Micrograms % vitamin A.

f. Precautions

(1) $SbCl_3$ must contain no moisture. (Keep stoppered when not in use.)

(2) Sample must be dry before addition of $SbCl_3$.

(3) Cleaning glassware: soak glassware containing $SbCl_3$ in a 10 percent solution of sodium potassium tartrate, rinse, and wash with detergent as usual.

(4) If no blue color is observed in the unknown tube (density less than .04), then the sample contains less than 14 micrograms percent vitamin A. If more than 25 percent of the group of samples give readings of less than 0.04, the size of the plasma sample should be increased to 3.0 ml.

(5) When pipetting off the petroleum ether extract, *do not* get any of the protein particles from the wall of the tube.

g. Standardization

(1) Carotene—Solutions of crystalline β-carotene may be used. The $E\frac{1\%}{1\text{ cm}}$ for carotene in hexane, at 452 mμ, is 2550.

(2) Vitamin A—U.S.P. standard vitamin A capsule may be used as directed. Alternatively, crystalline vitamin A acetate may be employed. The $E\frac{1\%}{1\text{ cm}}$ for vitamin A acetate in ethanol at 328 mμ, is 1730 (results expressed as vitamin A alcohol).

(3) With the reagents, volumes, and cuvettes given in the above procedure, with the Coleman Jr. Spectrophotometer, the constant for carotene and vitamin A is uniform from one instrument to another. The factors are:

(*a*) Carotene: D450 × 7.28 = micrograms carotene per ml. petroleum ether.

(*b*) Carotene correction: One microgram of carotene is equivalent to 0.017 density units at 620 mμ.

(*c*) Vitamin A: D620 (corrected) × 4.81 = micrograms vitamin A per tube.

h. Equivalents[1]

(1) One International Unit (I.U.) or United States Pharmacopoeia Unit (U.S.P.) of vitamin A is equivalent to 0.3 microgram of vitamin A alcohol. Thus,

International Units of vitamin A × 0.3 = micrograms of vitamin A.

(2) One International Unit (I.U.) or United States Pharmacopoeia Unit (U.S.P.) of provitamin A is equivalent to 0.6 microgram of β-carotene. Thus,

International Units of Provitamin A Activity × 0.6 = micrograms of β-carotene.

[1] *Reference:* British Journal of Nutrition. *5* 104 (1951).

5. Thiamine in Urine

REFERENCES

Modified from that described by Consolazio, Johnson, and Marek, Metabolic Methods, St. Louis, C. V. Mosby and Company, 1951.

Other pertinent references are:

Hennessey, D. J., and Cerecedo, L. R., J. Am. Chem. Soc., 61: 179, 1939.

Connor, R. T., and Straub, G. J., Ind. and Eng. Chem., Anal. Ed., 13: 385, 1941.

Mickelsen, O., Condiff, H., and Keys, A., J. Biol. Chem., 160: 361, 1945.

a. Principle

Thiamine is adsorbed from urine in acid solution by means of activated zeolite. It is eluted with concentrated potassium chloride, and is converted to thiochrome by ferricyanide in alkaline solution. Thiochrome is extracted into isobutyl alcohol and measured fluorometrically.

b. Apparatus

(1) Photofluorometer, Coleman model 12B or 12C and filters.

(2) Hennessey adsorption tubes with long (5 to 5.5 inch) stems.

(3) Fine glass wool.

(4) Reaction vessels. 40–50 ml. glass stoppered (interchangeable stoppers) vessels with a conical bottom.

(5) Cuvettes. Coleman 19 x 105 mm.

(6) Syringe pipettes—5 ml., 10 ml.

(7) Wooden applicators for inserting the glass wool into the columns.

c. Reagents

(1) Potassium chloride, 25 percent solution. Dissolve 500 gm. KCl in 1500 ml. 0.1 N HCl with the aid of heat. Filter.

(2) Bromcresol green indicator, 0.4 percent in 70 percent alcohol.

(3) Sodium acetate, 2.5 M. Dissolve 205 gm. anhydrous $NaC_2H_3O_2$ or 345 gm. of $NaC_2H_3O_2.3H_2O$ in water and dilute to 1 liter.

(4) Decalso. Wash 60 to 80 mesh Decalso 3 times with 3 percent acetic acid, once with 25 percent KCl, again with 3 percent acetic acid and then several times with distilled water. Each washing consists of stirring the Decalso 15 minutes in the wash, settling and decanting. Store wet in a stoppered bottle. Decalso which has already been activated may be purchased from Fisher Scientific Co., labeled "Thiochrome Decalso." Used Decalso may be re-activated.

(5) Sodium hydroxide, 1 N solution. Dissolve 40 gm. of sodium hydroxide in 1000 ml. of distilled water.

(6) Sodium sulfate, anhydrous, C. P.

(7) Acetic acid, 0.5 percent solution in water. Dilute 2.5 ml. glacial acetic acid to 500 ml. with distilled water.

(8) Isobutyl alcohol, redistilled. Collect the fraction between 105° and 108° C.

(9) Potassium ferricyanide, a 1 percent solution in water. This solution is stable for 6 months if kept in a dark bottle.

(10) Potassium hydroxide, a 15 percent solution in water.

(11) Oxidizing reagent. One part of 1 percent ferricyanide solution is mixed with 9 parts of potassium hydroxide (15 percent). Prepare fresh every day.

(12) Quinine sulfate standard:

(*a*) Stock standard. 100 mg. quinine sulfate per liter of 0.1 N H_2SO_4. Stable for 1 year.

(*b*) Intermediate standard. 25 ml. stock standard diluted to 100 ml. with 0.1 N H_2SO_4.

(*c*) Working standard. 5 ml. intermediate standard diluted to 500 ml. with 0.1 N H_2SO_4. Prepare daily.

(13) Stock Thiamine solution (100 mcg./ml.). Dissolve 50 mg. of dry thiamine hydrochloride in 200 ml. of 95 percent ethanol. Add 5 ml. 0.1 N HCl and bring to 500 ml. with distilled water. Store in refrigerator, stable for 6 months.

(*a*) Intermediate Thiamine solution. Dilute 5.0 ml. of stock thiamine solution to 100 ml. with water.

(*b*) Working Thiamine solution. Transfer 4.0 ml. of the intermediate solution to a flask containing 75 ml. of 0.1 N H_2SO_4 and 5 ml. of 2.5 N sodium acetate and adjust to 100 ml. Make fresh daily. The final concentration is 0.2 mcg. thiamine per ml.

d. Procedure

(1) Place a small piece of glass wool in the tip of the semimicro adsorption columns; fill the column with activated Decalso so that the bottom of the bell is just covered. Uniform filling is easily done by first filling the tube with water, then adding the Decalso and allowing to settle by gravity.

(2) Wash the column with 3 ml. 0.5 percent acetic acid and allow to drain.

(3) Take a 10 ml. aliquot of urine in a 50 ml. Erlenmeyer flask, add 2 drops of bromcresol green, and neutralize to a light greenish yellow color with 1 N NaOH or 1N H_2SO_4.

(4) Pour the sample onto the column and allow to drain by gravity into the waste pan.

(5) Rinse the flasks three times with 10 ml. of hot distilled water and put these washings through the column.

(6) Place a 50 ml. beaker under each column and elute successively with 10, 10, and 5 ml. of the acidic 25 percent KCl.

(7) Remove a 5 ml. aliquot from the beaker and place in the reaction vessel. Add 0.7 ml. of oxidizing agent and mix. Do not do more than 3 or 4 samples, at this step, at once as they must be shaken with isobutanol within *one minute* of the addition of the oxidizing agent. Remove another 5 ml. aliquot from the beaker and place in another reaction vessel for use as the blank. To this tube add 0.7 ml. of a mixture consisting of 1 part of H_2O mixed with 9 parts of 15 percent KOH. The time here is not critical.

(8) Then add 10 ml. isobutanol, stopper with the glass stopper and shake vigorously 50 times. After the layers have settled, the bottom layer is drawn off with suction.

(9) A small amount of anhydrous Na_2SO_4 is added and the vessel is tilted gently. The isobutanol layer must be clear after the sulfate has settled. If not, repeat this step.

(10) Repeat steps 3 to 9 using 10 ml. of working thiamine standard. Be sure to adjust the pH as in step 3. A reagent blank using 10 ml. of 0.5 percent acetic acid should also be run with each batch of reagents.

(11) The sample is then decanted into cuvettes to be read on the Coleman model 12C Photofluorometer.

(12) The B_1 filters are used and after the instrument has "warmed up" and has been balanced, the working quinine sulfate standard is used to set the machine at 60.

e. Calculation

$$\frac{\text{Unknown—Blank}}{\text{Standard}} \times \text{mcg. } B_1 \text{ in standard} \times 6 \text{ hr. urine volume} \times \frac{1}{2} =$$

mcg. B_1/6 hr.

$$\frac{U—B}{S} \times 0.4 \times \frac{6 \text{ hr. urine volume}}{2} = \text{mcg } B_1/6 \text{ hr.}$$

NOTE.—If the reagent blank is significant, this must be subtracted from the reading of the standard.

6. Riboflavin in Urine

REFERENCE

Connor, R. T., and Straub, G. J.: Combined Determination of Riboflavin and Thiamine in Food Products, Ind. and Eng. Chem., Anal. Ed. 13: 385–389 (June) 1941.

a. Principle [1]

Riboflavin is measured fluorometrically after interfering substances are destroyed. An internal standard is used, and the blank is determined after reduction of riboflavin to the leuko form, which is not fluorescent.

b. Apparatus

(1) Photofluorometer, Coleman model 12C, and B_2 and PC–2 filters.
(2) Cuvettes, Coleman 19 x 150 mm.
(3) Syringe pipettes, 1 ml., 3 ml.
(4) Graduated cylinders, 25 ml., with ground glass stoppers.
(5) Volumetric flasks, 100 and 1,000 ml.

c. Reagents

(1) Buffer solution, pH 4.7. Dissolve 111 gm. of sodium acetate plus 54.4 ml. of glacial acetic acid in water and dilute to 1,000 ml. Check pH.

(2) Potassium permanganate, a 4 percent solution in water. Store in dark bottle; 100 ml. is sufficient.

(3) Hydrogen peroxide, a 30 percent solution in water. Dilute to 3 percent for use. Keep refrigerated.

(4) Stock standard riboflavin solutions, 100 mcg./ml. Prepare by dissolving 10 mg. of standard riboflavin in exactly 100 ml. of 3 percent acetic acid. (3 ml. glacial acetic acid/100 ml.) Store in a dark bottle in a refrigerator. This solution is stable for 6 months.

(5) Working standard riboflavin solution: 1 mcg./ml. Dilute stock standard 1 to 100 with water.

[1] From Consolazio et al., *op. cit.*

(6) Sodium fluorescein reference standard. Dissolve 10 mg. of sodium fluorescein and dilute to 1,000 ml. with 3 percent acetic acid. For a working standard (0.5 mcg./ml.) dilute the stock 1 to 20 with water. (May need a 1:40 dilution, e.g., 0.25 mcg./ml.)

(7) Sodium hydrosulfite, C.P., powdered.

d. Procedure (To be done in duplicate)

(1) Pipette exactly 1 ml. filtered urine and 3 ml. buffer solution into a 25 ml. glass stoppered graduated cylinder or into a 10 ml. volumetric flask.

(2) Add 4 percent $KMnO_4$ drop by drop until a pink or violet color persists for one minute.

(3) Add dropwise H_2O_2 (3 percent) until the color disappears.

(4) Make up to 10 ml. with buffer solution, mix and transfer to cuvettes.

(5) Read samples within 1 hour as follows: (keeping in dark meanwhile)

(*a*) Warm up instrument for 15 minutes before using. Use B_2 filters.

(*b*) Balance at zero and set galvanometer at 60–80 with a sample of the dilute fluorescein reference in the cuvette. This setting should be kept constant for any one set of samples.

(*c*) Read the unknown (reading A).

(*d*) To the sample add exactly 1 ml. of standard B_2 solution (1.0 mcg. B_2).

(*e*) Mix thoroughly with stirring rod and read immediately (reading B).

(*f*) To the same cuvette now add a small amount of sodium hydrosulfite on the end of a spatula, stirring until dissolved.

(*g*) Read immediately (reading C).

e. Calculation [1]

(1) Reading A represents the galvanometer deflection due to riboflavin and other fluorescing materials in 10 ml.

Reading B represents the galvanometer deflection due to total fluorescence and added riboflavin in 11 ml.

Reading C represents the galvanometer reading due to the nonriboflavin fluorescence in 11 ml.

(2) Mcg. riboflavin/6 hr. urine =

$$\frac{A - \left(C \times \frac{11}{10}\right)}{\left(B \times \frac{11}{10}\right) - A} \times \text{mcg. riboflavin added} \times \frac{6 \text{ hr. urine volume}}{}$$

(3) Example

One ml. of urine was carried through the procedure and read 15.0 (reading A). After 1 mcg. riboflavin was added.

[1] From Consolazio et al., *op. cit.*

the reading was 50.0 (reading B). After hydrosulfite, the reading was 5.0 (reading C).

Mcg. riboflavin/400 ml. urine (for 6 hr.) =

$$\frac{15.0 - \left(5 \times \frac{11}{10}\right)}{\left(50.0 \times \frac{11}{10}\right) - 15.0} \times 1.0 \times 400 = 95.0 \text{ mcg.}$$

f. Precautions

(1) Keep samples in dark as much as possible.
(2) Because of the danger of reoxidation, read immediately after adding hydrosulfite.
(3) Keep sodium hydrosulfite stoppered when not in use.

7. N'Methylnicotinamide in Urine (as F^2)

REFERENCE

Huff, J. W., Perlzweig, W. A., and Tilden, M. W., Fed. Proc., 4: 92, 1945.

a. Principle

N'Methylnicotinamide reacts with acetone in an alkaline aqueous solution to produce a green fluorescent material. An excess of acid converts this compound into another more stable substance with a blue fluorescence, which may be measured with a photofluorometer.

b. Apparatus

(1) A photofluorometer, Coleman model 12C.
(2) Filters, same as for thiamine, B_1—PC_1.
(3) Test tubes, 10 ml. graduated or 15 ml. graduated centrifuge tubes.
(4) Pipettes, 1 ml. and 5 ml. graduated and 0.2 ml. serological.
(5) A water bath, boiling.
(6) Erlenmeyer flasks, 50 ml.
(7) Volumetric flasks, 25 ml.

c. Reagents

(1) Acetone, C. P.
(2) Sodium hydroxide, C. P., a 6 N solution.
(3) Hydrochloric acid, C. P., a 6 N solution.
(4) Potassium dihydrogen phosphate, C. P., a 20 percent solution.
(5) Quinine sulfate, USP reference standard. (Same as thiamine standard.)
(6) N'Methylnicotinamide Stock Standard:
   (*a*) Ten mg. of N'methylnicotinamide are dissolved in 100 ml. of water. This solution contains 100 mcg. per ml. and may be used for several months if kept in the refrigerator. If the iodide salt of N'MN is used calculations are made on N'MN ion.

   Dilute solution:

   (*b*) Two ml. of the stock standard are diluted to 100 ml. This solution contains two mcg. per ml. and is made fresh each day.
(7) Charcoal, activated.
(8) Acetic acid, glacial, C.P., a 2 percent solution.

d. Procedure

Every tenth sample should be done in duplicate to check reliability.

(1) Into graduated 10 ml. or 15 ml test tubes (centrifuge tubes) add the following solutions:

	Blank	Unknown	Recovery
Filtered urine	0.2ml	0.2ml	0.2ml
NMN diluted standard	0	0	0.4ml
Water	1.3ml	0.8ml	0.4ml
Acetone		0.5ml	0.5ml
6N/NaOH	0.2ml	0.2ml	0.2ml
Mix, wait 5 minutes and add 6NHC1	0.3ml	0.3ml	0.3ml

(2) All the tubes are mixed thoroughly and heated for 2 minutes in a boiling water bath and cooled in cold water.

(3) Add 1 ml. of the potassium dihydrogen phosphate solution and make up to 10 ml. with distilled water.

(4) Readings are made using filters B_1 and PC–1, and with the same quinine standard as for thiamine to adjust the instruments set at 60–80. All the tubes should be read at the same time and temperature (26°–30° C. is the steadiest range) and readings should be made quickly in order to avoid heating the tubes in the photofluorometer.

e. Calculations

$$\frac{\text{Unknown} - \text{Blank}}{\text{Recovery} - \text{Unknown}} \times 0.8 \times \frac{5}{1{,}000} \times 6 \text{ hr. vol.} = \text{mg./6 hr.}$$

g. Precautions

Highly pigmented urines low in N'methylnicotinamide and all urines with high blanks should be decolorized with charcoal (Malinckrodt's decolorizing charcoal or Merck's U.S.P. activated charcoal) by the following procedure before the determinations are made.

To 20 ml. of diluted urine in 2 percent acetic acid are added 35–50 mg. of charcoal; the flask is given a rapid swirl and the urine is filtered immediately.

V. Alternative Methods

(Acceptable substitutes for those listed in section III, 4 and 5, and IV)

1. Procedure for Drawing Blood Sample [1] (Vacutainer Technique)

a. Sterilization and Care of Needles and Adapters

Needles and adapters should be unplugged with the accompanying stylets. Check each needle for burrs and sharpness. If they need sharpening, use an oiled stone hone. Wash needles, stylets and adapters in soapy water and rinse thoroughly in distilled water. Insert the stylets into the needles. Sterilize the needles by placing in either a sterilizer or a boiling water bath for a minimum of 20 minutes. Store needles in

[1] If vacutainer method of collecting blood samples is used all blood determinations will be on serum instead of plasma.

alcohol, 70–80 percent by volume. Care must be taken to remove any alcohol from inside the needle prior to use.

b. Drawing Blood

(1) A table (6′ x 3′) is arranged so that the subject sits on a chair at one end with his right or left arm straightened over the table conveniently for one to draw blood from the antecubital vein. Racks containing the paired 5 and 20 ml. vacutainer tubes are placed near the subject's arms. Label each tube with masking tape. Dishes containing the sponges and needles in alcohol are placed conveniently. At the other end is a dish pan containing clean water. The area in the middle of the table is reserved for the adapters and extra vacutainer tubes.

(2) A crew of two men is engaged in these activities. The subjects are lined up in single file. Each man comes up in turn, sits in the chair, and straightens out his arm over the table. The assistant cleans and sterilizes the antecubital arm area and applies the tourniquet. Meanwhile, the operator screws the needle into the adapter; care must be taken to keep the sharp end sterile. He now takes the oxalated 5 ml. vacutainer tube and inserts it into the adapter allowing the dull end of the needle to pierce the rubber stopper until the proximal end is level with the mark on the adapter. The unit—tube, adapter and needle—is now one. The operator inserts the needle into a vein, and pushes the tube the rest of the way into the adapter. The vacuum will be broken and blood (approximately 4.5 ml.) will be sucked into the 5 ml. oxalated tube until the pressure is equalized. The tourniquet is released; the operator removes the tube, and the adapter and needle are left in place. Slight pressure with cotton gauze over the proximal end of the needle will control the flow of blood. Insert the 20 ml. vacutainer all the way into the adapter. Release the vein pressure and have the assistant retighten the tourniquet. Let the tube fill with blood (17–18 ml.). Remove the vacutainer tube, then the needle and adapter. A cotton gauze moistened with alcohol is placed in the antecubital space, and the subject is released with instructions to maintain firm pressure by hand for five minutes to prevent a hematoma.

(3) Remove the needle from the adapter and place both in cold water.

(4) The assistant numbers the labels on the tubes with *pencil.* The oxalate tube is inverted gently 20 times to thoroughly mix the blood immediately after its removal from the adapter. The 20 ml. tube containing whole clotted blood is immediately placed on ice. *Do not shake this tube or remove its stopper.*

From the oxalated blood the field determinations of total plasma protein, hemoglobin and hematocrit are performed as outlined in section IV of this chapter. The iced whole clotted blood specimens are packed upright in an iced thermos jug (13–17 per jug) and readied for shipment by the most rapid

means available to the main laboratory for the determinations of vitamins A and C and carotene. These tubes should be removed from the field laboratory to the main laboratory at least once a day.

c. Equipment for Blood Collection—Vacutainer Technique

(1) 5-ml. B. D. vacutainer 3204—oxalated KNH ..... ea.—110
20-ml. B. D. vacutainer 3208 ..... ea.—110
(2) Needles 20 gage—double ended vacutainers ..... ea.— 60
(3) Adapter for vacutainers and needles ..... ea.— 24
(4) Swabs (2″ x 2″) gauze, package of 100 ..... ea.— 3
(5) Basins—stainless steel—8-quart size ..... ea.— 3
(6) Brushes, test tube ..... ea.— 1
(7) Detergent, 100-gm. bottle ..... ea.— 1
(8) Evaporating dish, 200-ml. (for alcohol swabs) ..... ea.— 3
(9) Alcohol—95 percent, 1 pint ..... ea.— 1
(10) Alcohol—70 percent, 1 pint ..... ea.— 3
(11) Hematocrit tubes and caps ..... ea.—110
(12) Specific gravity bottles $CuSO_4$:
Sp. Gr. 1.018–1.033 (for plasma proteins) ..... 100 ml. ea.
Sp. Gr. 1.045–1.069 (for hemoglobin) ..... 100 ml. ea.
(13) Wooden applicators, box ..... ea.— 1
(14) Syringes:
1 ml. ..... ea.— 12
5 ml. ..... ea.— 4
10 ml. ..... ea.— 6
(15) Needles:
No. 15 gage—4 inches ..... ea.— 6
No. 22 gage—1 inch ..... ea.— 4
No. 22 gage—6 inch (for hematocrit) ..... ea.— 4
(16) Wire test tube racks, 40-hole ..... ea.— 6
(17) Wire basket 6″ x 6″ (partitioned) ..... ea.— 2
(18) Syringe pipette, 5-ml ..... ea.— 1
(19) Syringe, serological, 10-ml ..... ea.— 6
(20) Insulated food cans, 4-gal. (for refrigeration) ..... ea.— 2
(21) Wide mouth thermos bottles—2 quart (for shipping blood) ..... ea.— 8
(22) Masking tape, 2-inch roll ..... ea.— 2
(23) Interval timer ..... ea.— 1
(24) Rubber tubing for tourniquet ..... ea.— 4
(25) Lantern, large—2 spare batteries ..... ea.— 1
(26) Honing stone, light oil can—2-oz. size (for sharpening needles) ... ea.— 1
(27) Saline, physiological, 250-ml. bottle ..... ea.— 1
(28) Towels, lab. ..... ea.— 10
(29) Clinical centrifuges (transformer if required) ..... ea.— 2
(30) Sterilizer (transformer if required) ..... ea.— 1
(31) Stove, gasoline, 2-burner ..... ea.— 1
(32) Tongs ..... ea.— 1
(33) Forceps ..... ea.— 2
(34) Laboratory Coats ..... ea.—as required
(35) Cleansing tissue—small size box ..... ea.— 1
(36) Pencils—No. 2 ..... ea.— 6
(37) Tablets, writing, 8″ x 11″ ..... ea.— 2
(38) Carbon paper, 8½″ x 11″ sheet ..... ea.— 6
(39) Laboratory Book—for recording raw data ..... ea.— 1
(40) Wide mouth jars, 8-oz. for food samples ..... ea.— 12
(41) Twine ball ..... ea.— 1
(42) Pencil, red wax ..... ea.— 3
(43) Ice, dry or natural ..... as required

2. Plasma or Serum Vitamin C (Ascorbic Acid)—Dye Method

REFERENCES

Bessey, O. A., J. Biol. Chem., 126: 771, 1938.
Mindlin, R. L., and Butler, A. M., J. Biol. Chem., 122: 673, 1938.
Methods of Vitamin Assay, p. 148.

a. Principle

This method is based on the measurement of the extent to which the indophenol dye is decolorized by ascorbic acid in biological fluids. Since reduction of the dye is instantaneous but reduction of dye with interferring reducing substance is slow, the decrease in color intensity with time is determined, permitting correction for reduction of the dye by substances other than ascorbic acid.

b. Storage

This method estimates reduced vitamin C. Clotted whole blood collected in a vacutainer and held at refrigerator temperature may be safely stored for up to at least 48 hours prior to separation of serum and completion of the estimation. Once the serum is separated, however, it cannot be stored for more than a few hours, even though precipitated, without serious loss of ascorbic acid.

c. Apparatus

(1) A quantity of 100-ml. volumetric flasks.
(2) An analytical balance.
(3) A quantity of 10-ml. volumetric flasks.
(4) Volumetric flasks, 200-ml.
(5) Filter paper, Whatman No. 1.
(6) Paraffin.
(7) Bottles, polyethylene, 500-ml.
(8) Crucible, Gooch.
(9) An electric centrifuge.
(10) Stirring rods.
(11) Coleman, Jr. Spectrophotometer.
(12) Cuvettes, 19 x 150 mm.
(13) Centrifuge tubes, 15-ml.
(14) Syringe pipettes, 1 ml., 2 ml., 3 ml., 4 ml., and 5 ml.

d. Reagents

(1) All reagents should meet ACS specifications or be of reagent grade.
(2) Redistilled water (distilled in glass and free from copper) or deionized water must be used for all solutions.
(3) Metaphosphoric acid solutions:
   (*a*) Metaphosphoric acid, a 6 percent solution.
   Dissolve 6 gm. metaphosphoric acid pellets (34–36 percent HPO_3 by assay) without heating in redistilled water and make up to a volume of 100 ml. Store in the refrigerator. A fresh solution should be made every 10 days.
   (*b*) Metaphosphoric acid, 3 percent solution.
   Add 5 ml. 6 percent metaphosphoric acid to a 10 ml. volumetric flask. Make up to volume with redistilled water. Prepare this solution daily.

(4) Vitamin C Standard:

Dissolve 100 mg. ascorbic acid in 3 percent HPO_3 in 100 ml. volumetric flask and dilute to 100 ml. Dilute 1 ml. to 100 ml. with 3 percent HPO_3 (10 mcg./per ml.); dilute 20 ml. of the latter with 20 ml. 3 percent HPO_3 (5 mcg./ml.). Use 1, 2, 3 and 4 ml. for preparation of standard curve, make to 4 ml. with 3 percent HPO_3.

(5) Solution of 2, 6-Dichloro-benzenone-indophenol (sodium salt):

(*a*) Stock dye solution 50 mg./100 ml.

Dissolve 100 mg. 2, 6-dichloro-benzenone-indophenol Na salt in about 150 ml. of boiling redistilled water which contains 42 mg. of $NaHCO_3$. Cool to room temperature and make up to a volume of 200 ml. with redistilled water. Filter through No. 1 Whatman filter paper and store solution in a brown bottle in the refrigerator. Renew solution every 4 weeks.

(*b*) Working solution 2 mg. percent.

Dilute 4 ml. of stock solution to 100 ml. with redistilled water. Make this solution daily.

(6) Sodium hydroxide solutions:

(*a*) Saturated NaOH.

Dissolve 200 gm. of C.P. sodium hydroxide in 200 ml. of redistilled, boiled water in a pyrex flask. Allow to stand several days to permit the precipitation of sodium carbonate. Decant the supernatant solution and store the carbonate-free NaOH in a paraffin-lined bottle or a polyethylene bottle. If desired, instead of waiting several days for the precipitate to settle, one may centrifuge the solution or filter it through a Gooch crucible.

(*b*) A 2 N NaOH solution.

Weigh out 32.0 gm. saturated NaOH into an Erlenmeyer flask. Transfer to a 200 ml. volumetric flask and make up to the mark with boiled, cooled, redistilled water. Do not expose the solution to air any longer than necessary.

(7) Citrate Buffer:

Dissolve 29.4 gm. citric acid (monohydrate) in 140 ml. of 2 N NaOH (carbonate free) and make up to a volume of 250 ml. with redistilled water. Store the majority of this solution in the freezer to prevent mold growth. Small amounts of the solution may be stored in the refrigerator for several days, but before using this solution check it carefully to see that there is no mold growth which would cause turbidity.

e. Procedure

(1) In an 18 ml. screw cap test tube place 12 ml. of 6 percent metaphosphoric acid.

(2) Add exactly 4 ml. of plasma or serum and mix well with a stirring rod. Allow to stand at least 10 minutes or overnight if desired (cap and refrigerate before storage).

(3) Filter the supernatant fluid through No. 40 Whatman filter paper.

(4) Pipette exactly 4 ml. of the filtrate into each of two cuvettes, and for the blank, pipette 4 ml. of 3 percent metaphosphoric acid into two other cuvettes.

(5) To all the tubes add 1 ml. of the citrate buffer.

(6) Set the spectrophotometer at 515 mμ and zero optical density with distilled water as the blank, to obtain the center reading.

(7) Add 4 ml. of "working" dye solution to the blank quickly with a syringe pipette and shake quickly. Exactly 15 seconds after the addition of the dye, (start counting time after last drop of dye has been added) obtain a reading (D_1).

(8) Add a crystal of ascorbic acid to decolorize completely the dye and obtain a second reading (D_2). Repeat this procedure for the unknown samples.

f. Calculations

(1) Subtract the density of the solution after decolorization (D_2) from the value obtained before decolorization (D_1). This (D_3) represents the density value corrected for turbidity.

(2) Subtract this value for the *sample* from the corresponding value for the reagent blank. This value will be referred to as density difference.

(3) Determine the ascorbic acid concentration of the plasma or serum by referring to the chart prepared from the ascorbic acid standard curve.

g. Checks on the Method

(1) After the addition of the ascorbic acid to the blank tube, the reading should be 0.015 or lower. If it is not, a new dye solution should be made. Sometimes a particular batch of dye is at fault. Then discard dye and order new batch.

(2) The blank reading at 15 seconds should be around 0.350. If the reading falls much lower than that, some error probably occurred in the preparation of the dye solution, or the solution decomposed on standing.

VI. Elective Biochemical Methods

These procedures may be employed as time and other observations permit. The laboratory director should, however, instruct the personnel in these procedures.

1. Vitamin Tolerance Tests [1]

a. Principle

A dose of vitamins is administered and the urine is collected thereafter for 4 hours. If the body is well saturated with vitamins a considerable percentage of the test dose is excreted. If the body is unsaturated the vitamins are absorbed by the tissues and the urinary excretion is small.

b. Apparatus (assuming 50 subjects)

(1) A 2-gallon coffee pot or other container from which pouring is easy.

[1] Based on Consolazio et al., *loc. cit.*

(2) A large spoon for stirring.
(3) 50 paper cups, 8-oz.
(4) Apparatus for collecting and storing urine as previously described.

c. Reagents

(1) Water soluble tablets of thiamine hydrochloride, 5 mg. each.
(2) Water soluble tablets of riboflavin, 5 mg. each.
(3) Water soluble tablets of nicotinamide, 50 mg. each.
(4) Water soluble tablets of ascorbic acid, 100 mg. each.
(5) Chemicals required for collection and storing urine.

d. Procedure

(1) Allow at least 2 hours for mixing the solutions before administration of the dose.
(2) For each subject expected add 150 ml. of water to the pot, and 1 tablet each of thiamine, riboflavin and nicotinamide. Stir until dissolved.
(3) Before administration add for each subject 5 tablets of vitamin C. Stir until dissolved. Add the pills just in time to insure complete solution; ½ hour is usually enough except in cold weather.
(4) On a convenient table place one cup for each subject and pour 150 ml. of solution. It is well to calibrate the cups with a heavy pencil line at the 150 ml. mark.
(5) Immediately after the voiding the subjects are given the vitamin test dose in the presence of an observer.
(6) The doses of vitamins are so large that the subjects may now be allowed to eat breakfast without interfering with the test.
(7) All subjects should be briefed with instructions that the urine for the next 4 hours is to be collected.
(8) Exactly 4 hours after the administration of the vitamins the specimens of urine should be collected, measured, and stored as described in section III.

e. Calculations

Determine how much of the dose of vitamins is excreted in 4 hours.

f. Precautions

(1) The solution of vitamins must be protected from direct sunlight at all times because of the sensitivity of riboflavin.
(2) Vitamin C is so unstable that it must be added only just before administration.
(3) Very strict supervision is required to insure that the subjects drink all the vitamins and also that they collect all of their urine.
(4) The load test described above is a necessary compromise between practical conditions in the field and scientific desirability. In particular a longer collection period would be desirable owing to the relatively slow excretion of certain of the vitamins. Many workers feel that intravenous injection is far preferable to oral administration.

(5) It is advisable to buy vitamins in large batches. *It is essential to assay each batch before use* since commercial tablets usually contain an excess beyond labeled potency.

(6) For every 50 subjects it is a good idea to make enough solution for 5 extra doses in case of spillage or inaccurate measuring.

2. Therapeutic Trials

It is often possible to establish the identity of a deficiency syndrome or to differentiate between two similar appearing conditions through the use of a properly designed therapeutic trial. For instance, the etiologic role of a deficiency of niacin, iron or riboflavin in the production of glossitis may be ascertained by observing the effect of specific therapy on the glossitis while maintaining the patient in an otherwise unchanged environment. Such trials of therapeutic response should be so controlled that the diet of the individual is unaltered and care must be taken that other therapy is not simultaneously administered. The subject should be given therapeutically effective doses of a single nutrient under study for sufficient period to permit the occurrence of a result. It is well, if possible, to obtain biochemical or other evidence that the supplement has actually been ingested or where feasible to give the nutrient parenterally. It is desirable to design such tests to include groups of subjects who receive the nutrient(s) under study and another group who receive a similar appearing placebo.

The use of a placebo group is essential when the administration of the supplement is to be over a period of more than a few days in order to judge the expected changes which occur spontaneously. These latter changes may be particularly confusing if evaluation continues throughout a change in season or of weather.

The assessment of subjective phenomena in patients is especially hazardous and must always be critically designed with the use of the "double blind" placebo—i.e., a design in which the physician-observer as well as patient are ignorant of which individuals are receiving the placebo and which are given the therapy. Indeed, in any large scale or extended therapeutic trial it is advisable to employ the "double blind" design.

Properly utilized, the therapeutic trial serves as an excellent confirmatory or differentiating device. Of similar value also is the more extended investigation by ordinary medical procedures of typical cases of a syndrome which is encountered in the population. For example, a more precise study of a few cases of anemia through more detailed hematologic investigation, including bone marrow aspirations, will aid materially in establishing the nutritional relationship, if any, of the condition.

3. Creatinine in Urine (Picrate Method)

REFERENCE

Folin, O., and Wu, H., J. Biol. Chem., 38: 98–100, 1919.

a. Principle

Creatinine reacts with picrate in alkaline solutions at room temperature, and in a few minutes a stable intense orange color is produced which can be measured spectrophotometrically.

b. Apparatus

(1) Coleman Jr. Spectrophotometer, model 6.

(2) Cuvettes 19 x 150 mm.

(3) 100-ml. volumetric flasks.

(4) Calibrated syringe pipettes, 1, 2, 4, and 10 ml.

(5) Volumetric pipettes of 20-ml. capacity.

c. Reagents

(1) Sodium hydroxide, a 10 percent solution in water.

(2) Picric acid, a 1 percent solution in water. Gentle heating may be required to dissolve the acid. Mix 100 ml. NaOH and 100 ml. picric acid and dilute to 1000 ml. This solution is called the "alkaline picrate" solution.

(3) Crystalline creatinine zinc chloride. Dissolve and dilute 1.6106 gm. to 1000 ml. in 0.1 N hydrochloric acid. This solution contains 1.0 mg. of creatinine per ml.

e. Procedure

(1) Into a 100-ml. volumetric flask measure accurately 0.1 ml. of urine with a 0.1 ml. serological pipette.

(2) Dilute to approximately 20 ml. with distilled water and add exactly 20 ml. of the mixed sodium hydroxide and picric acid mixture.

(3) Allow to stand for exactly 15 minutes, after a gentle mixing, and then dilute to 100-ml. mark.

(4) A blank using exactly 20 ml. of the alkaline picrate mixture is set up and diluted to 100 ml.

(5) An aliquot (10 ml.) of the blank is pipetted into a cuvette and set at zero density at a wave length of 520 mμ.

(6) Then read aliquots of the unknowns.

f. Calculation

(1) Prepare a standard curve from creatinine zinc chloride solution:

(*a*) Dilute exactly 5 ml. of standard to 100 ml. This gives a solution of 0.05 mg. creatinine per ml.

(*b*) Into 100 ml. of volumetric flasks pipette 0, 1, 2, 3, 4, and 5 ml. of the dilute standard.

(*c*) Add 20, 19, 18, 17, 16 and 15 ml. of water respectively.

(*d*) Add exactly 20 ml. of the same alkaline picrate used for analysis of unknowns.

(*e*) Stand exactly 15 minutes and then dilute to 100 ml.

(*f*) Set blank at zero density and read all standards at 520 mμ.

(2) Plot the concentration-density curve on semilog paper.

g. Precautions

Many variables affect the intensity of the creatinine picrate. Important among these are: time of standing; temperature; concentration of alkali; and presence of noncreatinine color producing substances. Therefore, it is essential to establish standard conditions and to adhere strictly to these.

4. Tryptophan Load Test—Xanthurenic Acid in Urine

(Test for Vitamin B_6 Deficiency)

REFERENCES

Vilter, et al., J. Lab. Clin. Med., 42: 335, 1953.
Greenberg, et al., Arch. Biochem., 21: 237, 1949.
Wachstein and Gudaitis, Amer. J. Clin. Path., 22: 652, 1952.

a. Principle

It has been shown experimentally in adults, and under "normal" feeding conditions in infants, that a deficiency of vitamin B_6 promptly results in the excretion in the urine of an abnormal metabolite of tryptophan, xanthurenic acid. Whereas, normal individuals excrete very little xanthurenic acid (less than 50 mg.) in 24 hours, after a test dose of tryptophan in a deficiency of vitamin B_6, the excretion increases to 100–500 mg.

b. Load Test

A preliminary 24-hour urine collection is made. Toluene or oxalic acid may be used as preservative. The subject is then given 10 gm. of DL-Tryptophan (or 5 gm. of L-Tryptophan) in water and another 24-hour urine collected. Meals may be taken as usual.

c. Analysis for Xanthurenic Acid

(1) *Reagents*

(*a*) 0.4 M. tris (hydroxymethyl) amino methane buffer pH 7.8: dissolve 58 gm. of maleic acid (C.P.) and 60.6 gm. of tris in 500 ml. H_2O. About 4 gm. charcoal is added. The mixture is then shaken, and after standing 10 minutes, is filtered. 48.4 ml. of 1N NaOH is added to 40 ml. of the filtrate, and after diluting to 100 ml. with H_2O the pH is checked and adjusted if necessary. Buffer is stable in icebox.

(*b*) 1.7 percent Fe NH_4 $(SO_4)_2$. $12H_2O$ (0.85 gm. in 50 ml. H_2O).

(*c*) 60 mg. pure xanthurenic acid (XA) in 100 ml. ethanol. The XA is brought into solution by drop-wise addition of 1N NH_4OH.

(2) *Procedure*

(*a*) Place 2 ml. of filtered urine and 8 ml. H_2O in a test tube. More urine can be used if only small amounts of XA are present.

(*b*) Add 10 ml. buffer and mix by inversion.

(*c*) Pipette 10 ml. to a cuvette marked "unknown" and pour the remainder to another cuvette marked "blank."

(*d*) To the "unknown" add 0.1 ml. of 1.7 percent Fe NH_4 $(SO_4)_2$ and shake.

(*e*) Let stand 5 minutes.

(*f*) Set the colorimeter at 610 mμ; adjust to zero O.D. with the "blank." Read unknown solution.

(3) *Calculation*

From the standard curve, calculate the mg. xanthurenic acid excreted in 24 hours.

(4) *Comments*

Urine preserved with toluene can be kept in the icebox without loss of XA. The color of XA with the reagent is stable for several hours. Although pyridoxine deficiency has never been reported in adults under normal feeding conditions, the tryptophan load test would appear to be indicated in cases where other B-vitamins are known to be deficient. If B_6 deficiency is indicated as a result of the tryptophan load test, the oral admin-

istration of 15 mg. pyridoxine daily for 7 days should reduce the xanthurenic acid excretion, following tryptophan, to almost normal amounts.

5. Total Plasma or Serum Cholesterol

REFERENCES

Pearson, S., Stern S., and McGavack, T. H., J. Clin. Endocrin. and Metab., 12: 1245–6, 1952.

Pearson, S., Stern, S., and McGavack, T. H., Anal. Chem., 25: 813, 1953.

a. Principle

This procedure utilizes a modified Liebermann-Burchard reaction, which consists of treating plasma or serum with acetic acid, p-toluenesulfonic acid, acetic anhydride and sulfuric acid. The resultant greenish-blue color is measured spectrophotometrically.

b. Apparatus

(1) Cuvettes, 10 x 75 mm.
(2) Spectrophotometer, Coleman, Jr. 6.
(3) An interval timer.
(4) Syringe pipettes, 0.1, 0.5 ml. and 1.5 ml.
(5) Graduated 1-ml. pipette.

c. Reagents

(1) Glacial acetic acid, C.P.
(2) P-Toluenesulfonic acid, a 12 percent solution in glacial acetic acid.
(3) Acetic anhydride, C.P.
(4) Sulfuric acid, C.P. conc.
(5) Cholesterol standard, 200 mg./100 ml. of solution.

d. Procedure

(1) Pipette exactly 0.1 ml. of plasma or serum into a 10 x 75 mm. cuvette.
(2) In order, add 0.1 ml. of acetic acid, 0.5 ml. of p-toluenesulfonic acid and 1.5 ml. of acetic anhydride.
(3) Be very cautious and do not stir or mix.
(4) Allow the mixture to stand at room temperature until it cools and then add 0.2 ml. of sulfuric acid.
(5) After the addition of the acid, immediately shake the cuvette vigorously, until the precipitate is completely dissolved.
(6) Allow to stand at room temperature for exactly 20 minutes (this time interval is very essential) and measure the optical density at 550 mμ.
(7) To 0.1 ml. of the standard solution, add 0.1 ml. of distilled water, 0.5 ml. of the p-toluenesulfonic acid solution, and 1.5 ml. of acetic anhydride. After cooling add 0.2 ml. of sulfuric acid.
(8) Adjustment of zero point of the spectrophotometer
The solution with which the zero point of the spectrophotometer is set before measuring the optical densities of the standards and sera is prepared by substituting 0.1 ml. of water for serum in step 2, with the addition of all the other reagents.

(9) Preparation and reading of blanks

Blanks to correct for interfering substances in serum that absorb at 550 mμ are prepared by the addition of 2.3 ml. of p-toluenesulfonic acid solution to 0.1 ml. of serum. Set the zero point of the spectrophotometer with p-toluenesulfonic acid solution before reading the blank.

e. Calculation

$$\text{Mg cholesterol/100 ml. of plasma or serum} = \frac{100\ (a-b)d}{c}$$

Where:

a is the optical density of plasma or serum.
b is the optical density of blank.
c is the optical density of standard.
d is mg cholesterol/ml. standard solution.

f. Precautions

(1) It is very necessary *not to stir* the mixture after the addition of acetic acid, p-toluenesulfonic acid and acetic anhydride.

(2) A recent article in Clinical Chemistry (4: 345, 1955) Jones, B. J., and Moreland, F. B., describes an explosion in the use of p-toluenesulfonic acid that contained excessive water. They recommend setting the tubes in the rack under a shelf or covered with a board immediately after the addition of a new batch of p-toluenesulfonic acid and glacial acetic acid. One new batch of p-toluenesulfonic acid exploded even though there was no mixing.

g. Comments

(1) A simple direct procedure for the determination of total cholesterol in plasma or serum has been developed to meet the needs of a large-scale study of endocrinologic changes and lipid metabolism in old age. The procedure requires only the addition of reagents to serum and the measurement of the intensity of the resultant color. The determination is complete in less than 30 minutes.

(2) Plasma or sera were analyzed by this rapid procedure and by the classic Schoenheimer-Sperry method. An average deviation of +3.5 percent was found between the results obtained by the two methods.

(3) By this method, normal values for blood cholesterol of 170 to 240 mg. per 100 ml. of serum have been found. In patients suffering from hypothyroidism, arteriosclerosis, the nephrotic stage of chronic glomerulonephritis, or biliary obstruction, blood cholesterol concentrations from 297 to 1,193 mg. per 100 ml. have been obtained.

6. Plasma or Serum Albumin (Biuret Method) [1]

REFERENCE

Wolfson, W. Q., Am. J. Clin. Path., 18: 723–730, 1948.

a. Principle

The albumin and the three major globulin fractions are determined after precipitation, and measured colorimetrically in a spectrophotometer

[1] Based largely on Consolazio et al., *op. cit.*

by the addition of an alkaline copper sulfate reagent (biuret) which produces a bluish-purple color.

b. Apparatus

(1) Cuvettes, 19 x 150 mm.
(2) Coleman Jr. Spectrophotometer model 6.
(3) Centrifuge tubes, 15-ml. round-bottom.
(4) Centrifuge tubes, 15-ml. conical.
(5) Centrifuge, electric.
(6) Incubator set at 37° C.
(7) Calibrated 0.1, 0.2, 2.4, 4.9 and 9.8 ml. syringe pipettes.
(8) Rubber stoppers, No. 1, solid.

c. Reagents

(1) 23 percent sodium sulfate: dissolve exactly 23 gm. of anhydrous sodium sulfate in distilled water at 37° C. Make up to 100 ml. and keep in an incubator at 37° C.

(2) 28 percent sodium sulfite solution: dissolve exactly 28 gm. in water at 28° C. Shake, dissolve, and make up to 100 ml.

(3) Saline ammonium sulfate: in a liter volumetric flask dissolve 193 gm. of ammonium sulfate in approximately 500 ml. of water. Add 40 gm. of sodium chloride, dissolve and make up to 1,000 ml. with water.

(4) Biuret reagent (Weichselbaum, T. E., Am. J. Clin. Path., 16: 40, Tech. Sec. 10, 1946): dissolve 90 gm. of Rochelle salts in about 400 ml. of 0.2 N NaOH. Add 10 gm. of $CuSO_4 \cdot 5H_2O$. When dissolved, add 10 gm. of potassium iodide and make up to 2,000 ml. with 0.2 N NaOH. Store in rubber stoppered waxed glass bottle.

(5) Span-ether reagent:
1 ml. Span 20 } —Filter through fast filter paper and
99 ml. Ether (USP) } dilute to 100 ml. with ether.

(6) Ether USP.

d. Procedure for Plasma or Serum Albumin

(1) Pipette exactly 4.9 ml. of sodium sulfite solution into a 15-ml. round-bottom tube.
(2) Add exactly 0.1 ml. of serum and mix thoroughly by inversion.
(3) Add about 1 ml. Span-ether reagent, stopper, and invert gently for 30 seconds. Do not shake. Centrifuge as above.
(4) Pipette exactly 3.0 ml. of the clear centrifugate into a cuvette.
(5) Add 3 ml. of biuret reagent and mix well by inversion. Prepare a blank by using 3 ml. sodium sulfite plus 3 ml. biuret reagent.
(6) Let stand 30 minutes and read at 540 mμ, setting the blank at zero density.
(7) For a standard, use a plasma or serum albumin concentration of 25 gm. per 100 ml. in saline.

e. Precautions

(1) In the albumin determination, do not shake the tube vigorously. In some cases up to 30 percent of the albumin is lost in this way.
(2) This method depends on exact pipetting; therefore, syringe pipettes are used throughout the procedure.

(3) The plasma or serum albumin standard must be analyzed by the Kjeldahl method for exact analysis.

VII. Vitamin Reference Standards

1. Vitamin B_1 (Thiamine Hydrochloride)

REFERENCE

Specifications and standards may be obtained from U.S.P. Reference Standards, 46 Park Avenue, New York 16, N. Y.

a. Description

It occurs as small white crystals, or as a crystalline powder having a slight characteristic odor. When exposed to air, the anhydrous product rapidly absorbs about 4 percent of water. Its solutions are acid to litmus paper.

b. Solubility

One gm. of thiamine hydrochloride dissolves in about 1 ml. of water, and in about 100 ml. of alcohol. It is soluble in glycerin and is insoluble in ether and in benzene.

c. Assay

Weigh accurately from 50–60 mg. of the dried thiamine hydrochloride obtained in the test for loss on drying. (Dry about 500 mg. of thiamine hydrochloride accurately weighed at 105° C. for 2 hours. It loses not more than 5 percent of its weight.) Dissolve it in sufficient 0.1 N sulfuric acid to make 1,000 ml., and mix well. Dilute an aliquot of this solution, equivalent to 500 mcg. of thiamine hydrochloride with water to make 500 ml., and mix well. Use exactly 2 ml. of this assay solution and 2 ml. of the thiamine hydrochloride standard solution. (From a portion of the stock solution that has been warmed to room temperature, transfer to a 100 ml. volumetric flask an aliquot containing exactly 100 mcg. of thiamine hydrochloride, and dilute to 100 ml. with water adjusted to a pH of 3.5 to 4.3 with hydrochloric acid. Each ml. of this solution contains 1 mcg. of thiamine hydrochloride.) The fluorescence of the assay solution corresponds to not less than 98 percent of the fluorescence of the equivalent amount of the USP thiamine hydrochloride standard solution.

d. Packaging and Storage

Preserve thiamine hydrochloride in tight, light-resistant containers.

2. Riboflavin

a. Description

Riboflavin is a yellow to orange-yellow, crystalline powder, having a slight odor. It melts at about 280° C. and its saturated solution is neutral to litmus. When dry it is not appreciably affected by diffused light, but in solution, especially in the presence of alkaline, it deteriorates quite rapidly, the deterioration being accelerated by light.

b. Solubility

One gm. of riboflavin dissolves in from 3,000 to about 15,000 ml. of water, the variations in the solubility being due to difference in the internal crystalline structure of the riboflavin. It is more soluble in

isotonic sodium chloride solution, and less soluble in alcohol than in water. It is insoluble in ether and in chloroform but very soluble in dilute solutions of alkalis.

c. Packaging and Storage

Preserve riboflavin in tight, light-resistant containers.

d. Procedures for Making Stock Solution

(1) Standard riboflavin stock solution I.—Dissolve 50 mg. of USP riboflavin reference standard previously dried at 105° C. for 2 hours and store in the dark in a dessicator over phosphorus pentoxide, in 0.02 N acetic acid to make 500 ml. Store under toluene in a refrigerator. Each ml. represent 100 mcg. of USP riboflavin reference standard.

(2) Standard riboflavin stock solution II.—To 100 ml. of stock solution I add 0.02 N acetic acid to make 1,000 ml. Store under toluene in a refrigerator. Each ml. represents 10 mcg. of USP riboflavin reference standard.

(3) Standard riboflavin solution.—Dilute 10 ml. of stock solution II with water to make 100 ml. Each ml. represents 1.0 mcg. of USP riboflavin reference standard. Prepare fresh standard solution for each assay.

e. Assay Procedure

To each of four or more tubes (or reaction vessels) add 10 ml. of the test solution of the material to be assayed. To each of two or more of these tubes add 1.0 ml. of the standard riboflavin solution and mix, and to each of two or more of the remaining tubes add 1.0 ml. of water and mix. To each tube add 1.0 ml. of glacial acetic acid, mix, add with mixing 0.5 ml of potassium permanganate solution (1 in 25) and allow to stand for 2 minutes. Then to each tube add, with mixing, 0.5 ml. of hydrogen peroxide solution, whereupon the permanganate color must be destroyed within 10 seconds. Shake the tubes vigorously until excess oxygen is expelled. If gas bubbles remain on the sides of the tubes after foaming has ceased, remove the bubbles by tipping the tubes so that the solution flows slowly from end to end.

In the fluorometer, measure the fluorescence of the test solution containing 1.0 ml. of added standard riboflavin solution and call this reading A. Measure the fluorescence of the test solution containing 1.0 ml. of added water and call this reading B. Then to reduce the riboflavin present, add, with mixing, 20 mg. of sodium hydrosulfite to 2 or more tubes, measure the fluorescence within 5 seconds and call this reading C. Calculate results as previously indicated in this chapter.

3. Vitamin C (Ascorbic Acid)

a. Description

Ascorbic acid occurs as white or slightly yellow crystal or powder. It is odorless, and on exposure to light it gradually darkens. In the dry state, ascorbic acid is reasonably stable in air, but in solution it rapidly deteriorates in the presence of air. It melts at about 190° C. Its solutions are acid to litmus paper.

b. Solubility

One gm. of ascorbic acid dissolves in about 3 ml. of water and in about 30 ml. of alcohol; it is insoluble in chloroform, in ether and in benzene.

c. Assay

Dissolve about 400 mg. of ascorbic acid, previously dried at 105° for 1 hour and accurately weighed, in a mixture of 100 ml. of recently boiled and cool water and 25 ml. of diluted sulfuric acid and titrate the solution at once with 0.1 N iodine, adding a few drops of starch solution as the end point is neared. Each ml. of 0.1 N iodine is equivalent to 8.806 mg. of $C_6H_8O_6$ (ascorbic acid).

d. Packaging and Storage

Preserve ascorbic acid in tight, light-resistant containers.

4. Niacinamide

a. Description

Niacinamide occurs as a white crystalline powder. It is odorless or nearly so, and has a bitter taste. Its solutions are neutral to litmus paper.

b. Solubility

One gm. of niacinamide dissolves in about 1 ml. of water, in about 1.5 ml. of alcohol and in about 10 ml. of glycerin.

c. Assay

Place about 300 mg. of niacinamide, previously dried over sulfuric acid for 4 hours and accurately weighed, in a 500 ml. Kjeldahl flask, dissolve it in 200 ml. of water and add 50 ml. of a 40 percent solution of sodium hydroxide. Connect the flask by means of a distillation tray to a well-cooled condenser which dips into a vessel containing 40 ml. of boric acid solution (1 in 25). Boil gently for 20 minutes, avoiding as far as possible distilling any of the liquid. Then increase the temperature and distill about 200 ml. Cool the flask, add 75 ml. of water, and continue the distillation, collecting an additional 70 ml. of distillate in the same receiver. Add a few drops of methyl red solution to the liquid in the receiving vessel and titrate with 0.1 N sulfuric acid. Perform a blank determination with the same quantities of reagents and in the same manner, and make any necessary corrections. Each ml. of 0.1 N sulfuric acid is equivalent to 12.21 mg. of $C_6H_6N_2O$ (niacinamide).

d. Packaging and Storage

Preserve niacinamide in tight container.

5. Vitamin A

REFERENCE

Sebrell and Harris, ed., The Vitamins, Academic Press, Inc., New York, vol. I., p. 99, 1954.

Standards

The International Standard for vitamin A is a cottonseed-oil solution containing 3.44 mg. of crystalline vitamin A acetate in each gram, to which has been assigned a potency of 10,000 International Units. Thus, one unit of vitamin A is biologically equivalent to 0.344 mcg. of vitamin A acetate and chemically equivalent to 0.300 mcg. of vitamin A alcohol. The standard vitamin A acetate shall have a melting point of 57.8–59° C. and a light absorption at 325 mμ in isopropanol with an $E\frac{1\%}{1cm.}$ of 1,525.

The International Unit and the U.S.P. unit are identical.

For provitamin A activity, the standard is crystalline beta-carotene in oil. One International Unit is equivalent to 0.6 mcg. of beta-carotene. The standard β-carotene shall have a melting point of 180° C. (corrected) and show a light absorption at 465 mμ in benzene with an $E\frac{1\%}{1cm.}$ of 2,290, or at 455 mμ in cyclohexane with an $E\frac{1\%}{1cm.}$ of 2,400.

VIII. Instrumentation [1]

1. General Principles of Spectrophotometry

a. Transmission

The ratio of the intensity of the exit beam to that of the incident beam is called the transmission of the tube.

$$\text{Transmission} = \frac{B}{A} \tag{1}$$

Transmission is an expression of the total effect of the intercepting transparent body on the light beam, including the reflection losses as well as the actual absorption of the body itself. Thus transmission is a proper factor for expressing the performance of a light filter, since it is a measure of its total performance. It is not a term ordinarily used in spectrochemistry because the companion expression, transmittance, is much more convenient. Both transmission and transmittance are mentioned here in order that they may be recognized as different measurements and not regarded as different terms for the same measurement.

b. Transmittance

Suppose now that a test tube of water is further modified by the addition thereto of a portion "C" of a soluble colorant. The intensity of the exit beam will of course fall from the original value, B_r, to some lower value, B_s. The ratio of the intensity of this exit beam with "C" added to that with "C" absent is called the transmittance (T) of "C".

$$T_c = \frac{B_s}{B_r} \tag{2}$$

It is very important to notice that "T_c" is an expression dependent only on the nature and amount, "C," of the added constituent, independent of reflection losses and of absorption losses from all other constituents of the solution.

In spectrochemical procedures this independence of measurements is achieved by the use of optically identical cuvettes. One of these is filled with a solution that is either free from or else contains a known proportion of the considered constituent. This cuvette of solution is called the "reference." Another of the identical cuvettes is filled with the solution under test, containing an unknown proportion of constituent but otherwise optically identical with the reference. This latter is termed the "sample." The spectrophotometer is used to determine the difference in concentration, "C," between the reference and sample by measuring the relative intensities of the exit beam first with the reference and then with the sample in position.

It should now be clearly understood that we are always dealing with the transmittance of the difference in concentration of a constituent

[1] From Consolazio et al., *loc. cit.*

between reference and sample and not with absolute concentration. It will be evident also that transmittance measurements are based on the premise of optical identity, and this fact must be given due consideration in the selection and handling of cuvettes and in deciding on the proper reference solution.

c. Selecting the Proper Reference Solution

In clinical chemistry the reference solution will usually consist of a "blank" identical in composition with the sample except for the concentration of the considered constituent. In some instances distilled water may prove an acceptable blank but its use should be the exception rather than the rule because the possibility of introducing light absorbing constituents with reagents is usually very real and the slight gain in convenience seems hardly worth the cost. It is certain that a reference, consisting of all ingredients, possibly including a known proportion of the considered constituents, is by far the safest selection and, as explained above, it is perfectly permissible to regard the considered constituent "*C*" as the difference in concentration between reference and sample. The practice of employing an air reference is to be condemned.

In the development of new procedures as well as in the routine applications of existing methods too much stress cannot be placed on the very obvious but frequently overlooked fact that no spectrochemical analysis can be more reliable than its reference.

d. Color and Wave Length

Referring again to a cuvette of colorant the transmittance (T) of this solution will be less than that of pure water because it allows less light to pass. But let us assume further that the colorant is hemoglobin. Why does the solution appear red when viewed by white light?

"White" is actually a mixture of light rays of many different colors and only when these colors are combined in the proper proportions does the light appear white. Thus, when this cuvette of solution is viewed against white light it appears red because the hemoglobin has the peculiar ability to stop the blue and green light rays much more effectively than the red. The exit beam thus contains a much greater proportion of red light and so appears colored red. This property of a substance to interfere unequally with the passage of different colored light is termed selective absorption.

It will now be apparent that, because of selective absorption, the T of a colored solution will depend on the color of the light used for the measurement and that the presence of a constituent will be most evident when the T measurement is made with light of that color most strongly absorbed by the constituent. This then is the outstanding advantage of the spectrophotometer as compared to a colorimeter: that color most strongly absorbed by the constituent being measured can be readily determined and the sensitivity and reliability of the analysis greatly improved by then operating at this wave length, the relative effect of interfering constituents decreasing accordingly.

With a filter photometer or colorimeter it is customary to express the color of the light used for the T measurement by a name, such as "blue-green," or by an arbitrary number designating the type of filter used to produce the colored light. But such terms are only approximate color expressions and for absorption spectrochemistry it is both more

convenient and more exact to express the color of the light in terms of wave length (λ). This numerical expression of color is particularly convenient because it makes possible a graphical expression of the relationship between λ S and T, and this spectral-transmittance (S–T) curve strikingly indicates that particular wave length where the considered constituent absorbs most strongly and can most readily be measured.

The wave length scale of most spectrophotometers is calibrated in millimicrons (1 millimicron = 10 Angstrom units = 10^{-6} millimeter), and the wave length of the measuring light is controlled by adjusting the λ knob of the instrument until the required wave length appears at the index of the λ dial. The beginner should acquaint himself with the relationship between color and wave length by placing a white paper in the instrument's cuvette well and then noting the color of the light projected on this paper with various settings of the λ dial.

e. Preparation of Spectral-Transmittance Curves

The development of the spectral-transmittance (S–T) curve of the considered ingredient should be one of the first steps in the rational development of any new spectrochemical method. Obviously all such measurements should be made in an environment identical with that contemplated for the analysis and it need hardly be added that the final method must incorporate control measures for pH or other environmental factors that can affect the color characteristics of the considered constituent or of any other constituents of the solution on which the spectrophotometric measurements will depend. Usually the initial study will contemplate also the S–T curves of possible interfering constituents, and with such a group of curves before him the analyst can then intelligently select that wave length where the proposed method will be most sensitive to the concentration of the constituent being measured and least sensitive to interference.

The S–T curve is also of great importance in deciding the optimum wave length because, if the photometric measurements are made with monochromatic light having the same wave length as the characteristic absorption band of the considered constituent, the data will almost invariably obey the Lambert-Beer law with precision. Indeed, it is only when measurements are made with such monochromatic light that this law may be expected to hold exactly and, while exact observance is not imperative to exact analysis, it certainly is both convenient and reassuring. With properly developed and applied S–T curves, it will be found possible in almost every instance to select a proper wave length and so regulate the chemistry of the analytical method that the Lambert-Beer law applies well within clinical precision.

There do occur instances when it may prove desirable to work at some wave length other than that at which the considered constituent has maximum absorption and here again the S–T curve is a chart from which one may reach logical conclusions concerning the optimum wave length value. An example of this sort is the spectrochemical measurements of pH, using the dye methyl orange. If the beginner is not already familiar with the S–T curves of the acid and basic forms of this indicator it is suggested that he prepare them, using the same concentration in each instance. Then note that the wave length of maximum pH sensitivity does not coincide with either point of maximum absorption. Because the preparation of the S–T curves is so simple, and their sig-

nificance so fundamental and applicable, every operator should familiarize himself with their use by examining several typical colored solutions, preparing their *S–T* curves and then deciding the wave length best suited for the spectrochemical measurement of the colored constituent in each instance.

The *S–T* curve is prepared by measuring the transmittance of a single sample at a series of wave lengths within the range of the spectrophotometer, using a suitable reference. The data are then plotted on cross-section paper, conveniently with transmittance (T) as ordinate and expressed as percent, and with wave length (λ) as abscissa and expressed as millimicrons ($m\mu$). Usually the points first plotted will disclose regions of low T values and additional points will then be located in these regions until the minima are established to the desired exactitude. Most spectrophotometers are capable in most instances of indicating a minimum with an accuracy of 1 or 2 $m\mu$, simply by taking a series of closely spaced T readings in the region of the critical value.

The principal purpose of the *S–T* curve is to indicate that wave length at which the considered constituent has minimum transmittance and the exactness of this indication is highly dependent on the amount of the constituent that the measuring beam is caused to penetrate. If the sample solution is too dilute, or the cuvette too small, the amount of constituent intercepting the light beam may be insufficient to produce an exactly measurable response. Conversely, if the sample is too concentrated, or the cuvette too wide, the *S–T* curve may be so flattened at the region of minimum transmittance that the optimum wave length cannot be exactly ascertained. It is important to remember that the curve will be most indicative when the sample concentration and depth are so adjusted that the minimum T value will fall between 5 to 90 percent. Thus, good practice demands that the first step in the preparation of the *S–T* curve be the selection of a suitable cuvette size and solution concentration.

This brief discussion of *S–T* curves is presented primarily because their significance is too little recognized or at least too little utilized. Actually, one of the outstanding advantages of the spectrophotometer is that it allows the development of these curves and thereby a simple, intelligent approach to the development of new spectrochemical methods and a more exact application of old ones.

f. The Lambert-Beer Law

The absorption of light by a substance is due to the transmission of the individual atoms or molecules from one energy state to another. The spectrophotometer is an instrument capable of measuring the intensity of light absorbed by a given substance at a given wave length, making possible identification and quantitative analysis of substances in solution.

The most satisfactory operation of spectrophotometers is with systems which obey Beer's law, dealing with light absorption. The law can be stated thus: (*a*) all layers of a solution, each of a given thickness, absorb the same fraction of light incident upon them; and (*b*) all layers of solution, each containing a given number of molecules, absorb the same fraction of light incident upon them. Thus the intensity of light absorbed depends both on concentration of solute, and length of light path through the solution.

If I is intensity of incident light,
dI is intensity of light absorbed,
dc is concentration in moles per liter, and
dl is the thickness of a layer

$$\frac{dI}{I} \propto dl, \text{ and } \frac{dI}{I} \propto dc$$

$$\int_{I}^{I_o} \frac{dI}{I} \propto c \int_{0}^{1} dl \quad \text{and} \quad \int_{I}^{I_o} \frac{dI}{I} \propto c \int_{0}^{c} dc$$

$$ln \frac{Io}{I} \propto 1 \qquad \text{and } ln \frac{Io}{I} \propto c$$

combining,

$$ln \frac{Io}{I} \propto lc \qquad \text{or } ln \frac{Io}{I} = klc$$

$$\text{or } \log \frac{Io}{I} = \epsilon lc \qquad \text{or } D = \epsilon lc \qquad \text{(equation 1)}$$

where D = optical density or $\log \frac{Io}{I}$,
ϵ = molar extinction coefficient, and
c = concentration in moles per liter

In this way, using the last equation, if D is determined experimentally for a solution of known concentration and lightpath at a given wave length the ϵ can be calculated. Since ϵ is independent of c and l when Beer's law holds, c for any other solution can be calculated from density readings. To test the applicability of Beer's law, optical densities of solutions of the same thickness (l) are plotted against concentration. Since $\epsilon \, l = \frac{D}{c}$, ϵ l should be the slope of the graph, which should be a straight line.

If it is desired to use transmittance instead of optical density, Equation 1 transforms into the following expression:

$$c = K \log 1/T \qquad \text{(equation 2)}$$

or more simply

$$c = -K \log T \qquad \text{(equation 3)}$$

In words, equation 3 states that the concentration of solute is proportional by a negative constant to the log of the transmittance.

g. The Concentration-Transmittance Calibration Graph

The importance of the Lambert-Beer law is stressed here first, because it is the most simple and, second, because it is the only rational means for translating photometer readings to expressions of the corresponding concentration of the sample. The law also frequently allows the simultaneous determination of two or even three different constituents in the same sample, without mutual interference.

The relationship between C (Concentration) and T (Transmittance) is exactly expressed by the Lambert-Beer law in practically every instance where the requirements of this law are fully met. These four requirements are:

(1) The T measurement must be made with monochromatic light.

(2) The T measurement must be made at a wave length corresponding to a region of the constituent's S–T curves where T is substantially constant, i.e., at the middle of a region of maximum absorption or on a "flat" portion of the curve. The measurement should not be made on the "side" of an absorption band except under unusual circumstances.

(3) The reference must be so selected that $C = 0$ when $T = 100$ percent.

(4) The nature of the sample solution must be such that its T responds only to changes in C.

Equation 3 significantly states that any concentration, C, of a given solution is proportional to the log of its corresponding transmittance, T. This means that the concentration-transmittance (C–T) graph of this relationship will be a straight line if plotted on semi-log coordinates, and such a C–T graph constitutes a calibration by which the concentration corresponding to any particular T value may be conveniently determined.

The equation further states that this specified straight line will always intersect the point ($C = 0$, $T = 100$ percent). Accordingly it is possible to prepare the C–T calibration graph of an analytical method without calculations. To do this, determine the T of one solution of known concentration, C, plot this known point on semi-log coordinates, and then draw a straight line intersecting this point and the point ($C = 0$, $T = 100$ percent). This line is the C–T calibration graph of the method.

The precision of this method of plotting will depend on how faithfully the analytical method follows the Lambert-Beer law, and it is always advisable to prepare and measure the T of several solutions of known concentration within the contemplated range of the method. When plotted, these several points should all fall close to the plotted straight line.

Let it be repeated: the technique of almost any determination can be so developed that the data follow the Lambert-Beer law with great precision, provided that measurements are made with a true spectrophotometer. When these conditions are met, the construction of the corresponding C–T calibration graph is fully as simple as it sounds.

h. Optical Density

It is sometimes convenient in spectrochemistry to express the light stopping ability of a sample in terms of its optical density, D, when

$$D = 2 - \log \text{percentage transmittance}$$

The advantage of this method of expression is that D values are directly proportional to corresponding C values. For example:

The optical density of a solution (let us say copper carbamate that has been extracted from serum) is designated as D ml., the serum blank as Ds, and the reagent blank as Dr. Copper concentration is obtained by the following calculation:

(1) K is determined from the calibration curve

(2) D ml. $- (Ds + Dr) = D\text{Cu}$

(3) $D\text{Cu} \times K \times 100 =$ Copper, mcg. per 100 ml.

i. Operation of Spectrophotometer, Coleman Jr., Model 6: See manual provided with instrument.

REFERENCES

Radley, J. A., and Grant, J., Fluorescence Analysis in Ultraviolet Light, 3rd edition, Chapman and Hall, Ltd., London, 1939.

Dake, H. C., and De Ment, J., Fluorescent Light and its Applications, Chemical Publishing Co., New York, 1941.

De Ment, J., Fluorescent Chemicals and their Application, Chemical Publishing Co., New York, 1942.

a. Definition

As the term is used in biochemical analysis, "fluorescence" means the emission of visible light by a substance when it is irradiated with ultraviolet light. If emission of visible light occurs after the removal of the ultraviolet light, the afterglow is termed "phosphorescence."

b. Laws of Fluorescence

Fluorometry is based on the general laws of photochemistry, and there are three specific "laws of fluorescence."

(1) Energy must be absorbed by a luminescent system before emission can occur (the absorption law of De Ment).

(2) In a fluorescent system the emitted wave lengths are longer than the absorbed wave lengths; that is, the energy emitted is less than the energy absorbed (the emission law of Stokes).

(3) The absorption of radiation by a luminescent system is a quantum process, involving one quantum per absorbing unit (e.g., atom or "center"), the yield being unity only in the ideal case (one aspect of the photochemical equivalence law of Einstein).

c. General Principles

All quantitative fluorometry is based on a fundamentally simple system. A source of ultraviolet light casts a beam on the solution to be examined. Visible light is emitted and is measured either visually (against a comparison standard) or photoelectrically.

In general, it is necessary to conduct fluorometry with dilute solutions, which are measured against calibration curves prepared under conditions identical with those of the analysis. The reason for this is that the absolute intensity of fluorescence is highly variable depending upon many important factors, including temperature, time of exposure, nature of instrument, traces of impurities, pH, and "quenching." "Quenching" is a complex phenomenon of which there are at least three kinds: (1) interference of a molecule's fluorescence by other organic molecules; (2) interference by inorganic molecules; (3) reabsorption of visible light by other molecules of the same compound as those being measured. Fluorometry by its nature is not as capable of extreme accuracy and reproducibility as is spectrophotometry. Every kind of analysis is strongly influenced by the nature, purity, history, and condition of the material under analysis.

Dietary Studies

I. Introduction

Dietary studies constitute an essential part of any complete nutrition survey. They involve collection of information concerning food habits, food supply, ration allowances, menu preparation, food procurement and distribution, food waste and the nutritional adequacy of the food issued and consumed. Dietary studies yield information on the adequacy of existing diets and the fundamental causes of any inadequacy. Although both clinical and biochemical studies provide direct means of measuring the nutritional status of individuals, the basic information collected in dietary studies is necessary in order to plan changes in nutritional practices as required. In addition, the same information must be available in order to modify existing economic, agricultural and food management policy and programs to assure the best use of available food supplies.

1. Objectives

The objectives of the dietary studies are to determine the following:

a. The average intake of essential nutrients per person per day.

b. Local food habits, the overall food supply, and cost of foodstuffs.

c. The official ration allowance if used and detailed information concerning planning and nutritional adequacy of menus.

d. The method of food procurement and distribution.

e. Nutritional adequacy of food issues during different seasons of the year.

f. Local messing and food service procedures.

g. Actual amounts of individual foods consumed, and loss or waste of individual foods.

2. Personnel

a. United States Team Members

Nutritionist member of the team 1
Messing specialist 1

b. Nationalist Cooperating Members (Minimum number)

(1) Officers:

Medical 1
Food service specialist (Quartermaster) 1
Biochemist or Veterinarian 1

(2) Enlisted Personnel:

Laboratory technicians or military mess specialists 4
Interpreter 1

3. Equipment and Supplies

a. Scales, double pan type (5-10 kgs. capacity, including set of weights).

b. Scales, platform type, Fairbanks-Morse, capacity 300 pounds, one-quarter pound interval.

c. Triple Beam balance capacity of 600 gm.

d. Automatic calculator, two slide rules.

e. Pencils, paper pads, etc.

f. Tables of food composition (preferably of native foods). FAO March 1954 Bulletin and U.S.D.A. Handbooks, No. 8, June 1950, and No. 34, March 1952. USDA Table of Food Composition for the Armed Forces.

g. Sample containers and shipping cartons for collecting samples of unknown foods to ship to analytical laboratory.

h. Hand tally counter, two.

i. Film and flash bulbs for camera.

j. Clip boards for record sheets (6 each).

k. Army Recipe Manual TM 10–412 and TM 8–501, Nutrition.

l. Kitchen spatulas.

m. Heat-resistant special ink for tare weights.

n. Plastic brief case or folders for records.

o. Waring Blendor.

II. Procedures for Military Dietary Surveys

1. Definition of Terms Used

a. "As purchased."—The form in which the food is issued to mess. Such as carcass beef, raw, untrimmed vegetables, etc.

b. "Nonedible refuse."—The portion of food that is not usually eaten, such as bones, fruit pits, rind and peel, egg shells and outer leaves of vegetables. In using food consumption tables based on "as purchased" in accordance with a specified percentage of inedible portion (refuse), if, due to cooking practices, the percentage differs from that noted, corrections should be made.

c. "Edible portion."—As purchased weight minus refuse weight.

d. "Plate waste."—Weighed amounts of plate scrapings separately by food item and separately as edible and nonedible portions.

e. "Kitchen waste."—Weighed amounts of food prepared but not served, *only* if discarded. Also food partially prepared but discarded, including cooking fats.

f. "Preparation loss."—All nonedible parts of food prepared that are discarded.

2. Ration Allowances and Menu Planning

The basic food allowance, and the "master menu" should be analyzed to determine the nutrient intake per man per day. When area menus are used due to availability of foods at varying locations, these should also be analyzed. Calculations should show the various foods and amounts planned per man per day, and the contribution of the nutrients from each food with appropriate references given for the values used in the computation. Also, estimates of possible nutrient loss during cooking should be calculated with references given on the loss figures used.

If monthly or quarterly menus are used, analysis should be made of menus over a sufficient period of time so as to include all major seasonal variations.

Most military forces have a monetary allowance which cannot be exceeded in the procurement of the ration. In some cases, this is based on specified amounts of individual food items authorized per man per day; while in others, no specific allowance of foods exists. Unless the monetary ration allowance or portion thereof is given directly to unit commanders for the direct procurement of food items, menus are prepared which determine to a large extent the food items procured and issued to the unit kitchens.

Sometimes, a master menu is prepared and subsequent area menus are made which embody only those changes considered necessary to render it most appropriate for each location in view of any differences in availability of food supply as well as local food habits of the troops. Complete information should be obtained concerning the basic food and monetary allowance, master menus and as many area menus as considered necessary during the year preceding and including the survey period.

3. Food Procurement and Distribution

Where centralized procurement and distribution of food items is employed, detailed information concerning the system must be obtained. Calculate the nutrient content of the ration "as issued" by Divisions and subordinate units for different seasons of the year. In many cases, certain items of food will be procured centrally for the entire military force while others, such as perishable vegetables, will be obtained locally through field buying offices. In other instances, unit commanders will be provided a portion of the monetary allowance for the ration and be charged with procuring certain perishable commodities.

Determination of nutritional adequacy of food issues to Division or subordinate units where this is practiced, provides exceedingly important information since this usually can be calculated from the supply records for different seasons of the year. This is done by selecting the appropriate units which are receiving the full ration and determining the average amount of the foods issued per man per day on the basis of unit strength reports for those periods.

From such data, the average nutrient composition of the ration as issued may be calculated. This method permits an appraisal of the nutritional adequacy of the ration for units at different times of the year when the available food supply might be quite different. Hence, one can obtain information for a longer period than is feasible with mess survey techniques.

4. Dietary Intake Studies

Prior to starting a dietary intake study in the unit kitchen, the team members should become familiar with the procedure of food procurement, food storage and issue, menu planning and monetary value of the ration. Throughout the survey, the procedure of food service should be further investigated. The report should contain a completed form, Form 1 ICNND, of the general food procurement and issue system in operation. Special attention should be given to the various types of rations, e.g., supplementary, hospital, and field.

Whenever possible, it is desirable to conduct the detailed dietary intake studies in units from which individuals are drawn for the detailed clinical and laboratory surveys. In any case, a typical, normal mess or kitchen is selected. A period of approximately five to seven days which

will cover a normal cycle of meals is desirable. However, in instances where little variety is found in the food supply, a shorter period is satisfactory.

A general description of primary food likes and dislikes of the troops should be made. A record of the number of men taking the various foods should be made. These data are to be obtained by observation during the meals.

Nutrient intake data can be obtained by three different methods which are described. These are the food inventory method, the food preparation and consumption method, and the food composite analysis method. The latter method is perhaps best used in conjunction with either of the first two.

a. Food Inventory Method

(1) The evening before the study is to start, a complete inventory of all food items in the mess hall (kitchen, storage tent, etc.) is made.

(2) When a considerable number of cooking pots are used it is usually desirable to weigh and mark all cooking vessels to be used in food preparation.

(3) During the period of the survey, all foods coming into the mess, including the daily issues of perishable foods and weekly issues of nonperishable items, are weighed and recorded. Foods should also be recorded as to the source of issue; that is, Quartermaster, local purchase, barter, etc.

(4) During the period of the survey, keep an accurate count of all men eating the food at each meal, either in or outside the mess, including regulars, casuals, cooks, etc. If three meals are served and the number of men eating varies from meal to meal, the total number fed at the three meals is divided by three to give the average number of rations for the day. Where more than three meals are fed, such as midmorning and afternoon teas, it is necessary to weight the meals according to their relative importance in obtaining the total number of rations for the period.

(5) A measure of the fat lost as waste grease and fat drippings *not subsequently consumed must be obtained.* Unless this is an appreciable amount, several determinations may be made of the small quantity of fat drippings and fat in plate washings; and this value may then be used in all subsequent computations. Nonedible kitchen waste such as vegetable peeling, bones, cores, and pits from fruit, etc., is weighed and recorded. When this contains proportions of potentially edible food, it should be separated and recorded as edible waste.

(6) During the hours of mess operation, one or more members of the survey team must be present to record recipes, method of preparation, cooking time, temperature, pH of cooking water, amount of water and fat used, seasoning, etc., as well as the weights of all issued foods utilized by the cooks as a check on the inventory method.

(7) During the survey, when there is evidence of food waste, all plate and kitchen *edible food* which is discarded, is collected, weighed and recorded by food item (see Form No. 4.

ICNND). This weight of the edible food waste must then be converted to the "as purchased" weight. This is done by use of the recipe and correcting for moisture gain or loss, fat loss, etc. (See example calculation, page 113.)

(8) During the collection of the dietary intake data, the team obtain and record the necessary data on ICNND forms Nos. 1 through 5, as required.

(9) After the supper meal of the last day of the study, another complete food inventory is made.

(10) Calculations to be made:

(*a*) Average weight of each food consumed per man per day. The gross quantities of food used for the period studied are calculated by subtracting the final inventory from the sum of the initial inventory amounts plus the daily issues. From these gross values are subtracted all edible food items wasted or not consumed after conversion to equivalent weights of the foods as issued. This yields the total of each food item consumed.

$$\text{Food item consumed} = (\text{Initial inventory} + \text{issues or purchases}) - (\text{final inventory} + \text{waste})$$

The average daily weight of each food item consumed per man per day, is then computed by dividing the total weight of each food item consumed by the sum of the number of days involved, times the average daily number of men consuming the food.

$$\text{Food item consumed per man per day} = \frac{\text{Total weight of food item consumed}}{\text{days of survey} \times \text{av. no. of men fed dy.}}$$

(*b*) The average nutrient consumption data are computed by the use of food composition data applicable. The United States Department of Agriculture Handbook No. 8 (June, 1950) and No. 34 (1952) and F.A.O. Bulletin, Food Composition Tables for International Use (March, 1954) are most helpful. In addition to these tables, values for food not listed and for vitamin losses during cooking in the local manner, should be obtained from the regional scientific literature or from local laboratories. Where unusual foods are encountered, they should be identified and described. If they contribute a significant portion of the ration, arrangements should be made to have samples forwarded to ICNND or to another laboratory for analyses.

(*c*) After the nutrient evaluation of the diet has been made, correction for losses of some of the heat labile or water soluble vitamins should be calculated. Local data on nutrient cooking losses should be used when available. References and corrections applied should be documented. (See section V this chapter.)

(*d*) Where extra food may be obtained outside the mess, an estimate as to the amounts and nutritional contributions should be made by visiting the nearby post exchanges, canteens, etc., and by questioning the men on food purchased in nearby towns.

(11) General Forms to be used:

(*a*) Survey of Food and Nutrition Services in the Armed Forces, form No. 1, ICNND.

(*b*) Food Inventory Record, form No. 2, ICNND.

(*c*) Recipe—Food Preparation, form No. 3, ICNND.

(*d*) Food Waste, form No. 4, ICNND.

(*e*) Summary—Nutrition evaluation, form No. 5, ICNND.

b. Food Preparation and Consumption Method

This method is very similar to the Food Inventory Method, except that the amount of food consumed is determined merely by weighing the amounts of each food item used in the preparation of each meal directly, rather than by difference, as is accomplished by the Food Inventory Method. The number of individuals consuming each meal is recorded and the average amount of each food item used in the preparation of each meal is determined for the survey period. From these data nutrient intake calculations are made in the same manner as described for the food inventory method.

This method is suitable particularly for kitchen operations that have little variety and relatively simple food preparation practices. This method also eliminates the need for survey personnel to record the food brought into, or removed from the mess hall.

c. Food Composite Analysis Method

For this method, the weight of the prepared food is obtained as it leaves the kitchen and the weight of any left-over prepared food, or plate waste not consumed, is determined. By dividing the number of men eating into the amount of prepared food consumed, the average amount of each item consumed per man per meal, is obtained.

At the same time, aliquots of each container of food prepared (two samples per container) are taken at each meal. It is desirable to obtain at least six or eight small aliquots of each food item served at the meal. These are combined and stored in covered containers on ice or under refrigeration until the end of the evening meal each day.

At that time, the various aliquots are homogenized in a Waring Blendor by food items and one-tenth of the amount consumed by the average man at each meal is accurately weighed to the nearest tenth of a gram on a triple beam balance. This is done for each item served at each meal.

The resulting homogenized aliquots for the various meals during the day, representing one-tenth of the amount consumed by the average individual, are then combined to form a composite sample for the entire day.

To this, 5 percent oxalic acid is added and the total weight is increased to 500 gms. or some other convenient number, by the addition of water.

At the end of the survey period, equal amounts of each day's composite food aliquot are then combined to form a master aliquot for the entire survey period. After the 5 percent oxalic acid has been added, these samples need not be refrigerated but should be stored in brown glass or plastic bottles with screw-type lids in order to minimize losses from oxidation and light, and prevent change in moisture content.

These samples are then analyzed locally for the critical nutrients of interest, or sent back to ICNND or to another laboratory for analysis.

This method of obtaining nutrient intake data is particularly desirable where unusual food items are employed in large quantities. It also

provides excellent information to serve as a check on the dietary intake data obtained by either of the other two methods described above. Furthermore samples may be subjected to assay for nutrients not generally considered critical, including various amino acids, vitamins and minerals.

III. Procedures for Civilian Dietary Studies

Obtaining reliable food and nutrient intake data in civilian populations is generally more difficult than in armed forces or institutional feeding situations, chiefly because the small family unit rather than a large mess unit is involved. Although the same procedures may be used, (e.g., inventory or daily prepared food aliquot analysis), these methods require fulltime participation on the part of survey team personnel for a number of days in each kitchen. Hence, in the case of small family units, these methods yield data on very few individuals for the time involved.

A further complication is apparent in the composition of the family and the different nutrient requirements for adults, children, infants and pregnant or lactating women, which makes it desirable to determine average nutrient intake for each of these categories separately.

Because of these factors, it has been necessary, in general, to depart from the methods mentioned above that give very precise information on a few individuals, and adopt procedures that are inherently less accurate but include large numbers of family groups.

In any civilian situation the same rules of courtesy and protocol apply that are required in the armed forces. The population must be approached through the local government and medical public health authorities, and it is necessary to enlist the cooperation and assistance of any and all civilian agencies related to social welfare, food rationing and distribution, etc.

1. Family Food Questionnaire and Record Procedure

In populations with an advanced social organization and educational level, housewives can be requested to fill in a detailed food use form which includes answers to questions on the age, sex, and food habits of each member of the family, and the amounts of food purchased during a weekly period. In some instances, this can include the use of a kitchen scale for the weighing of food portions by the housewife for each meal.

2. Recording of Actual Intake at Mealtime by Survey Personnel

This procedure requires a large number of volunteer workers, usually women. A preliminary training period is usually desirable. Sample areas are selected and the survey personnel assigned a specific number of homes. (See ICNND Forms Nos. 6 and 7.)

3. Dietary Histories

In areas where there is very little variety in food items, or severe food shortages, the skilled nutritionist can obtain a series of quite accurate daily dietary histories from housewives or cooks, in a relatively short time, if representative food items and several different sized portions of each are used as visual aids. This requires prior assessment of the food types and amounts available in the community and local food habits and customs.

4. Diets in Hospital and Other Institutions

Much vital information may be obtained through close contact and study of the hospitals, rest homes, out-patient clinics, orphan homes,

insane asylums, child-welfare centers and local physicians, regarding specific nutrition problems in special categories, such as the sick, the aged, and pregnant and lactating women—groups which are most sensitive to nutritional deficiency and where difficulties commonly occur before the family units are affected.

IV. Examples of Survey Forms

Form No. 1 ICNND: Survey of food and nutrition services in the Armed Forces

Date..... Location.....

a. General.—Describe and answer the following:

(1) Food procurement system.
(2) Finances of food procurement.
(3) Ration distribution and issue system.
(4) Food storage system.
(5) Are troops employed in food production, especially on a local basis? Extent?
(6) Are troops employed in food processing, locally?
(7) Food service supervision:
  (*a*) Schools for cooks and bakers? Manuals or training aids?
  (*b*) Mess hall—kitchen, preparation, serving.
  (*c*) Rations—extent to which scales are met.
  (*d*) Ration supplements and frequency.
  (*e*) Monetary allowance for ration? How used?
  (*f*) Welfare funds? Red Cross, etc.

b. Hospital and Medical

(1) Dietetic service in military hospitals, describe the following:
  (*a*) Type.
  (*b*) Adequacy of service.
  (*c*) Training of personnel.
(2) Is a manual used in feeding of sick and wounded?
(3) Status of nutrition training of medical staffs.
(4) Summarize reports and collect copies when available, (include source).
  (*a*) Heights, weights and other anthropometric data.
  (*b*) Surveys of nutrition.
  (*c*) Casualties and disabilities from malnutrition.
  (*d*) Special reports on nutrition problems.

c. Status of Civilian Nutrition (include source and data):

(1) Are dependents of soldiers consuming army ration? Secretly or openly?
(2) Is food from civilian sources coming into troops' hands?

d. Locally Determined Nutritional Requirements or Standards.—List report and summary data available on the following:

(1) Trainees.
(2) Combat.
(3) Bivouac or detention camps.
(4) Civilian.

e. Sanitation.—Check and describe the following:

(1) Cleansing of utensils and messing facilities.
(2) Water supply—source, treatment, etc.
(3) Disposal of wastage.
(4) Inspection of mess.

Form No. 2 ICNND: Food Inventory Record

Unit surveyed_____ Dates_____ Average men fed per day_____

Food item	Unit of issue	Unit weight (Lbs. or Kg.)	Initial inventory units	Units issued during period	Total issue units	Final inventory units	Total units used	Total used (Lbs. or Kg.)	Average per man per day "as issued" (Lbs. or Kg.)	Average edible waste per man per day	Average "as consumed" per man per day

Form No. 3 ICNND: Recipe—Food preparation

Unit .....
Day ..... Food — ....
Meal ..... Native Name United States Name

Ingredients	Weight as purchased	Refuse weight (percent)	Raw weight	Edible weight	Cooked weight	Conversion factor [1] Cooked weight to "as purchased"

Description of preparation: (Give actual recipe, including cooking time, method of cooking. Time prepared—time served, number of men for whom prepared, etc.).

Notes on Calculations

In both the (*a*) Food Inventory Method and the (*b*) Food Preparation and Consumption Method, it will be necessary at times to convert cooked foods to the "raw" or "as issued" basis and to determine the quantities of the ingredients in various cooked, mixed foods. For single food items, this can be calculated from the raw and cooked weights. For mixed dishes, the recipe giving the quantity (weight) of each item used and the final cooked weight must be obtained.

The following example illustrates the type of calculation which may be required:

Potatoes, hashed brown	Grams	Pounds	Percent in cooked product
Weight of unpeeled potatoes	36,350	80.07	
Weight of peeled potatoes	27,310	60.15	91.5
Weight of fat drippings	2,550	5.61	8.5
Total weight (uncooked)	29,860	65.76	
Total weight (cooked)	25,630	56.50	

[1] Note.—See sample calculation page 113.

Total amount cooked (25,630 gms.) was served.
Plate waste and leftovers discarded = 5,090 gms. or 11.21 lbs.
Raw equivalent is obtained by:
Total weight (uncooked), 29,860 × wt. of cooked discards, 5,090 = 5,930 gms. or
Total weight (cooked), 25,630 13.1 lbs.
Weight of raw, peeled potatoes discarded = 5,930 × .915 = 5,426 gms. or 11.9 lbs.
Weight of raw fat drippings discarded = 5,930 × .085 = 504 gms. or 1.1 lbs.
Factor for converting raw peeled potatoes to "as issued" is $\frac{36,350}{27,310}$ or 1.33.
Weight of potatoes (as issued, discarded) is 11.9 × 1.33 or 15.8 lbs.
Prepared dishes containing more ingredients are calculated in the same manner.

Form No. 4 ICNND: Food waste

Unit .....
Day ..... Number served.....
Meal .....

Edible Food

Food	Plate waste, total pounds (kgs.) food	Kitchen waste, total pounds (kgs.) food	Ingredient list (from recipe)	Conversion factor to "as purchased"	Total foods wasted, pounds (kgs.) as purchased
1					
2					

Total fat and grease discarded = ...

Form No. 5 ICNND: Summary—Nutritional evaluation

Unit ..... Physical activity .....

Date .....

Food	Quantity per man per day	Calories	Protein (gm.)	Carbohydrate (gm.)	Fat (gm.)	Calcium (gm.)	Iron (mg.)	Vitamin A (International Units)	Thiamine (mg.)		Riboflavin (mg.)		Niacin (mg.)		Vitamin C (mg.)	
									a	b	a	b	a	b	a	b
Total																

Footnotes:
a = no cooking losses considered.
b = corrected for estimated cooking losses (section V).

Form No. 6 ICNND: Family food record [1]

Date Recorded.....

Name..... Age.....

Home Province..... Religion.....

Number and ages of dependents:

Wife: Age..... Others:

Children: Boys.....Ages..... Adults.....Ages.....

Girls.....Ages..... Children....Ages.....

Total Family Income, Monthly..... Daily Food Cost.....

ONE-DAY RECORD

Menu for the day	Amounts—			Total quantity	Cost	Remarks: Snacks
	Bought	Home produced	Received free			
Breakfast:						
Lunch:						
Supper:						
Total.....						

[1] Reproduced by courtesy of the Philippine Institute of Nutrition.

Form No. 7 ICNND: Family food record—Food intake pattern

Food item	How often used				Remarks
Vegetables:					
Leafy and yellow					
Legumes					
Others					
Fruits:					
Citrus fruits					
Others					
Fat-Rich Foods:					
Butter or margarine					
Coconut					
Others					
Protein-Rich Foods:					
Milk					
Meat					
Poultry					
Fish					
Shellfish					
Liver					
Variety meats					
Dried beans					
Eggs					
Cheese					
Cereals & Tubers:					
Rice					
Corn					
Others					
Noodles, etc.					
Prepared desserts (cakes, ice cream, etc.)					
Candy					
Soft drinks					
Miscellaneous					

V. Average Percentage of Nutrients Lost During Cooking [1]

	Thiamine	Riboflavin	Niacin	Ascorbic acid
Meats	35	20	25	.....
Meats plus drippings	25	5	10	.....
Eggs	25	10	0	.....
Cereals	10	0	10	.....
Legumes	20	0	0	.....
Vegetables—leafy green and yellow	40	25	25	60
Tomatoes	5	5	5	15
Vegetables, other	25	15	25	60
Potatoes	40	25	25	60

[1] Based on good cooking practices with United States Army ration components. For individual foods in some of the food classes the loss may be considerably greater or less. These estimated average cooking losses are useful as guides but may not necessarily apply when cooking practices and conditions (e.g. pH) differ greatly. It is preferable to use local values where available and reliable. Department of the Army Technical Manual, TM 8-501, "Nutrition", September 1949, table II, p. 19.

Suggested Interpretive Guide

I. Introduction

This chapter includes suggested guides for interpretation of biochemical, dietary, and clinical data. (Height-weight standards are discussed in chapter 3.) These guides are not to be interpreted as standards or rigid nutritional allowances. The primary purpose for this chapter is to permit a more uniform interpretation of the data collected among the survey teams.

The general objectives of a nutrition survey are to determine the average dietary intake and the incidence of clinical signs (physical and biochemical) which are related to suboptimal nutrition. This combined approach to the problem gives more meaningful information than any one approach taken separately. It must be realized that dietary data represent only the situation at a given, limited time. Seasonal foods and other variations in dietary intake might alter significantly the nutritional status of the individual. The physical signs in conjunction with the biochemical findings represent the results of dietary habits of long duration, and provide a means for estimating the proportion of the population with suboptimal nutrition.

In interpreting the significance of the physical findings, there are several factors which must be kept in mind when dealing with relatively mild deficiency states. Almost any population group, even a well nourished one, will show a low incidence of most of the physical signs listed. Even though the average intake of a nutrient may be adequate, there will be individuals with higher requirements, or individuals who consume less than average amounts and thus are deficient or borderline with respect to the nutrient. Few, if any, of these physical signs in mild form are "specific" or "diagnostic" of a particular nutrient deficiency since they can be produced by nonnutritional factors or by any one of a group of nutrient deficiencies. Certain of the physical signs are looked for not because they have specific importance in predicting nutrient deficiency, but because they have some relationship to general health status, with possible secondary nutritional implications. Furthermore, it is known that physical signs of nutrient deficiencies come and go, often unpredictably, in mild deficiency states. In spite of these limitations, it is well established that the incidence and severity of these physical signs *in population groups* has a diagnostic significance as to the average nutritional state of the group, especially when considered collectively with dietary and biochemical data.

II. Biochemical

1. Suggested Guide to Interpretation of Urinary Vitamin Excretion Data—Adult Males

	Deficient	Low	Acceptable	High [1]
N'Methylnicotinamide:				
mg/6 hours	<0.2	0.2—0.59	0.6—1.6	>1.6
mg/gm creatinine	<0.5	0.5—1.59	1.6—4.3	>4.3
Riboflavin:				
mcg/6 hours	<10	10—29	30—100	>100
mcg/gm creatinine	<27	27—79	80—270	>270
Thiamine:				
mcg/6 hours	<10	10—24	25—50	>50
mcg/gm creatinine	<27	27—65	66—130	>130

[1] *High:* This term is used here in the sense of "high" for the prevention of recognizable clinical deficiencies or definite biochemical evidence of deficiency. Good or satisfactory would be advocated by many to replace this term. Nutrient consumption and biochemical values in the "high" range will be found in many countries which enjoy a high level of health and productivity. However, the precise health advantages which attend these "high" levels are the subject of much difference of opinion and little conclusive evidence.

The urinary values indicated above are based on an average creatinine coefficient of 23 and a 65 kg. man who would be expected to excrete 1.5 gm. of creatinine daily.

a. N'Methylnicotinamide

REFERENCES

Goldsmith, et al., J. Nutr., 56: 371, 1955.

Frazier, E., Prather M., and Hoene, E., J. Nutr., 56: 501, 1955.

Perlzweig, et al., J. Nutr., 40: 453, 1950.

Handler, Zeit. fur Vitaminforshung, 19: 393, 1948.

Unglaub, W. G., and Goldsmith, G. A., *in:* U. S. Quartermaster Food and Container Inst. for the Armed Forces, Chicago, Surveys of Progress on Military Subsistence Programs, Series II, No. 2: Methods for Evaluation of Nutritional Adequacy and Status, NAS-NRC, Washington, D. C., pp. 73–75, 1954.

N'Methylnicotinamide (NMN) and the 6-pyridone of N'methylnicotinamide (6–P) are the principal urinary niacin metabolites. Usually they are excreted in approximately equal amounts, although often there is slightly more 6–P. There is some evidence that the excretion of 6–P declines somewhat faster on a deficient diet than does NMN but this difference does not appear to be large and is not consistent. The assay procedure for 6–P is considerably more cumbersome than for NMN. Hence, only NMN assays are recommended.

It should be noted that some people in the post-absorptive state excrete little or no NMN. An unexpectedly large excretion of NMN is frequently observed in prolonged fasting, in patients with wasting diseases and in subjects in negative nitrogen balance.

b. Riboflavin

REFERENCES

Unglaub and Goldsmith *in:* Methods for Evaluation of Nutritional Adequacy and Status, pp. 72–73.

Goldsmith, G., Federation Proc., 8: 553, 1949.

Lowry, O. H., Physiol. Rev., 32: 431, 1952.

Horwitt, M. K., et al., J. Nutr., 41: 247, 1950.

Jolliffe, N., and Tung, T. C., Nutrition Status Survey of the Civilian Population of Formosa, Reprint from Metabolism, vol. 5, No. 3, pp. 309–327, May 1956.

Sinclair, H. M., Vitamins and Hormones, VI: 154, 1948.

Aykroyd, W. R., et al., Canad. Med. Assoc., 60: 1, 1949.

The urinary excretion of riboflavin is quite variable and caution must be exercised in interpreting results. Riboflavin output is increased in

acute starvation, in negative nitrogen balance, after certain antibiotics and in certain disease states.

The guide levels suggested would be considered low according to Jolliffe and Tung's (5) results in children, according to the Oxford standards (Sinclair, 1948) and the Newfoundland study (Aykroyd, 1949). These studies indicates that <200 mcg./gm. of creatinine would be an excretion level below which clinical signs of riboflavin deficiency could be expected. However, other studies conducted under metabolic ward conditions (Horwitt, 1950), analyses of random urine samples (Lowry, 1952), and other studies (Goldsmith, 1949), indicate somewhat lower levels. Since the Newfoundland study indicates that children excrete two to three times as much riboflavin as adults (when the results are expressed in terms of urinary creatinine), and since these guides are designed specifically for young adult males, the lower levels listed are recommended until experience may confirm or disprove them.

c. Thiamine

REFERENCES

Unglaub and Goldsmith *in:* Methods for Evaluation of Nutritional Adequacy and Status, pp. 69–71.
Jolliffe and Tung, *op. cit.*
Aykroyd, et al., *op. cit.*
Sinclair, *op. cit.*
Salcedo, J., et al., J. Nutr., 36: 561, 1948.
Louhi, H. A., et al., J. Nutr., 48: 297, 1952.
Burch, H. B., et al., J. Nutr., 46: 239, 1952.

The excretion of thiamine is to a considerable extent a characteristic of the individual. Thiamine excretion correlates well with dietary intake but not too well to deficiency states in individuals except at the very low ranges.

2. Suggested Guide to Interpretation of Blood Data
Young Adult Men

	Deficient	Low	Acceptable	High
Hemoglobin gms/100 ml.:				
Sea Level	<12.0	12.0—13.9	14.0—15.0	>15.0
5,000 ft. (1500 M.)	<12.3	12.3—14.2	14.3—15.6	>15.6
12,000 ft. (3700 M.)	<13.3	13.3—15.4	15.5—17.2	>17.2
14,000 ft. (4500 M.)	<14.4	14.4—16.7	16.8—19.0	>19.0
Hematocrit (PCV) in percent:				
Sea Level	<36	36—41	42-45	>45
5,000 ft. (1500 M.)	<38	38—43	44—47	>47
12,000 ft. (3700 M.)	<42	42—48	49—56	>56
14,000 ft. (4500 M.)	<46	46—52	53—65	>65
Total plasma protein: (TPP) [1] gms/100 ml	< 6.0	6.00—6.4	6.5—7.0	> 7.0
Plasma ascorbic acid: mg/100 ml	< 0.1	0.10—0.19	0.2—0.4	> 0.4
Plasma vitamin A: mcg/100 ml	<10	10—19	20—50	>50
Plasma carotene: mcg/100 ml	[2]	20—39	40—100	>100

[1] See notes on total plasma protein.
[2] See notes on carotene.

a. Hemoglobin and Hematocrit

REFERENCES

Sinclair, *op. cit.*
Bessey, O. A. and Lowry, O. H., Meals for Millions, New York State Joint Legislative Committee on Nutrition, Legislative Document No. 61, p. 175, 1947.
Wintrobe, M. M., Clinical Hematology, Lea and Febiger, 1942.
Hurtado, A., Merino, C., Delgado, E., Archives of Int. Med., 75: 284, 1945.
Cannan, R. K., Science, 122: 3158 p. 59, 1955.

The proposed guides are derived entirely from studies with Occidental races. Nutritional deficiencies should be suspected if more than 3 percent of a population group showed hemoglobins of 12 gm. percent or less at sea level. However, possible effects of customary altitude, parasitism, race, climate, etc., must be considered in interpreting results.

The standards are for adult men only. Adult women normally have hemoglobin levels 1 to 1.5 gm. lower than men. Children below age 14 also have hemoglobin levels 1 to 2 gm. below these levels.

According to Wintrobe, normal young males should show an average hemoglobin of 16.0 gm. (range 14–18) with an average hematocrit of 47 percent (a range of 40–54 is considered normal). However, most surveys of adult men have revealed an average hemoglobin in the range of 15–15.5 gm.

b. Total Plasma Protein

REFERENCES

Same as for Hemoglobin and Hematocrit.
Aykroyd, et al., *op. cit.*
Williams, H. H., et al., J. Am. Diet. Assoc., 27: 215, 1951.

It should be remembered that nutrition surveys of some malnourished population groups have shown normal total serum protein values but abnormal albumin-globulin ratios (e.g., an increase of globulin above normal). The levels specified assume a normal A/G ratio. If protein malnutrition is suspected in a population group with total serum protein in the normal range, measurement of serum albumin is indicated.

c. Plasma or Serum Ascorbic Acid

REFERENCES

References 1 and 2 under Hemoglobin.
Aykroyd, et al., *op. cit.*
Williams, et al., *op. cit.*
Clayton, M. M., et al., Bull. 516. Maine Agric. Exp. Station. Univ. of Maine, Orono, Maine, May, 1953.

A plasma or serum ascorbic acid level of <0.1 is compatible with a diagnosis of scurvy but not diagnostic. In clinical scurvy the level is usually about zero. Surgery, trauma, stress and many factors other than deficient diets may reduce serum ascorbic acid. Serum ascorbic acid does correlate fairly well with diet intake in groups of people, however. The level of ascorbic acid in leukocytes is a better indicator of clinical deficiency than is the plasma or serum level.

d. Plasma or Serum Vitamin A

REFERENCES

References 1 and 2 under Hemoglobin.
Aykroyd, et al., *op. cit.*
Williams, et al., *op. cit.*
Clayton, et al., *op. cit.*

There is little agreement on desirable levels of vitamin A. However, levels above 30 mcg. are usually accepted as representing good levels. Five percent of subjects with levels below 10 mcg. or 15 percent of subjects below 20 mcg. would suggest probable vitamin A deficiency.

e. Plasma or Serum Carotene

REFERENCES

References 1 and 2 under Hemoglobin.
Williams, et al., *op. cit.*
Clayton, et al., *op cit.*
Gillum, H., Morgan, A., and Sailer, F., J. Nutr., 55: 655, 1955.

No deficiency level of carotene can be specified although the plasma or serum level of carotene correlates very well with dietary intake but has no diagnostic significance as to vitamin A deficiency. The guides suggested must be regarded as being very tentative. They are considerably lower than recommended by Bessey and Lowry and by Sinclair, but fit fairly well with the data of Williams et al., and Clayton et al. On the other hand, Gillum et al., in presumably well nourished older men, found a distribution of carotene values from 25 to 405 mcg./100 ml. with means of 111–130.

III. Dietary

1. Suggested Guide to Interpretation of Nutrient Intake Data

	Deficient	Low	Acceptable	High
Niacin, mg/day	<5	5—9	10—15	>15
Riboflavin, mg/day	<0.7	0.7 —1.1	1.2—1.5	>1.5
Thiamine, mg/1000 Cal.	<0.2	0.20—0.29	0.3—0.5	>0.5
Ascorbic Acid, mg/day	<10	10—29	30—50	>50
Vitamin A, I.U./day	<2000	2000—3499	3500—5000	>5000
Calcium, gm/day	<0.3	0.30—0.39	0.4—0.8	>0.8
Iron, mg/day	<6.0	6—8	9—12	>12
Protein, gm/kg	<0.5	0.5 —0.9	1.0—1.5	>1.5
Calories				

These guides are intended to apply to 25-year-old physically active males of 67 inches (170 cm.) in height and 143 pounds (65 kg.) in weight living in a temperate climate and consuming a varied diet. The quantities specified should never be considered as inflexible "requirements." In interpreting nutrition surveys of population groups, average values falling in one or another of the above categories conceal the fact that some individuals will receive more and others less than average. In addition, it is known that there is much variability from one to another individual in their requirements for various nutrients. Variations in body size, activity, climate, types of food available, and other factors modify requirements and, consequently, interpretation of survey data (see notes for each nutrient).

The nutrient content of food may be altered materially during food preparation, a fact which must always be considered in evaluating dietary intake data.

a. Niacin

The requirement for niacin is usually about 10 times that of thiamine in both man and animals. Pellagra has been produced with diets containing less than 7.5 mg. of niacin (and very low in tryptophan) but pellagra has been prevented with diets supplying about 5 mg. of niacin per day when good sources of tryptophan were present. Evidence of tissue unsaturation has been found with diets supplying up to 8-10 mg. of niacin (and also low in tryptophan). It seems clear that both niacin and tryptophan in diets as well as the level and type of protein must be considered in estimating niacin adequacy. Sixty mg. of tryptophan can be considered as roughly equivalent to 1 mg. of niacin. The availability of niacin in various foods and composition of diets may also influence apparent niacin requirements.

b. Riboflavin

Riboflavin requirements are apparently not influenced by caloric intake or muscular activity. Clear-cut deficiencies have been produced experimentally with dietary intakes below 0.6 mg./day, possible signs of deficiency with intakes less than 0.9 mg. daily, and evidence of tissue unsaturation at levels below 1.3 mg. daily. The dietary requirement for riboflavin may be influenced by environmental factors such as sunlight, and by the efficiency of utilization of riboflavin from various types of foods.

c. Thiamine

Thiamine requirement is usually considered to be related to the total calories consumed. Clear-cut deficiencies have been produced with dietary intakes of less than 0.2 mg./1000 Cal. Evidence of tissue unsaturation has been found with intakes of 0.3 mg./1000 Cal. Intakes of 0.3–0.5 mg./1000 Cal. would be regarded as acceptable.

d. Ascorbic Acid

There seems little doubt that daily diet intakes of 10 mg./day of ascorbic acid, will prevent scurvy in most adults and that 30 mg./day is a reasonable level to prevent signs of scurvy. Whether higher dietary levels of ascorbic acid serve a useful function is an unsettled issue.

e. Vitamin A

Vitamin A requirement is proportional to body weight. Levels of intake which provide 20 International Units (6 mcg.) of preformed vitamin A per kg. of body weight daily, or 40 International Units (24 mcg.) of beta-carotene will meet minimal requirements. The values in the table represent vitamin A "activity" and assume a diet providing ⅓ preformed vitamin A and ⅔ as beta-carotene. The availability of carotene in different types of foods varies considerably, and its utilization may be influenced by the other dietary constituents such as the amount of fat.

f. Calcium

Calcium balance can be attained with intakes of as little as 0.3 gm. daily. Adaptation to low calcium (and to high calcium) diets is a well-known phenomenon. Diet intake figures must be evaluated in the light of traditional diet and evidence of calcium inadequacy such as bone demineralization. In evaluating calcium intakes, consideration must be given to sources of calcium such as drinking water, salt and calcium compounds used in processing foods.

g. Iron

In normal males, the daily loss of iron from all sources approximates 1 mg. Since the efficiency of absorption of dietary iron is usually less than 10 percent, a daily intake of 9-12 mg. should be realistic. There is excellent evidence that normal males can be maintained on much lower intakes. However, in dealing with groups where parasitism may be high, and where the need to regenerate blood may be a matter of considerable practical importance (in military forces), the need for dietary iron may be increased.

h. Protein

An intake of 1 gm. of protein per kg. of body weight should be regarded as a practical and realistic minimum when dealing with popula-

tions where a high proportion of the protein will come from nonanimal sources. Cereal proteins are often deficient in certain essential amino acids, especially lysine, methionine and perhaps threonine. Nitrogen balance and health, insofar as it can be measured, can be maintained on strict vegetarian diets if the quantity and variety of protein is sufficient. It is well known that nitrogen balance can be attained with diets providing as little as 20-30 gm. of protein daily. However, nitrogen balance does not necessarily reflect all the functions of protein in maintaining normal metabolism and health. The level of protein in diets should provide some margin for variations in individual requirements and to facilitate repletion of protein stores after nitrogen loss due to injury, disease or temporary diet inadequacies. The protein levels specified assume that caloric needs have been met.

i. Calories

The only certain method of assessing caloric adequacy is to measure the number of calories necessary to maintain desired body weight and necessary levels of physical activity over an extended period. A young male of the indicated height, weight and age, living in temperate climate (mean annual external temperature 10° C.), who is fairly active physically, being neither sedentary nor engaged in hard physical labor, and who is assumed to engage in a moderate amount of outdoor recreation, will probably require about 3,200 calories. This level is probably excessive for urban "white collar" workers.

Calorie requirements are influenced by several factors for which adjustments can be calculated.

(1) *Body Size*

Calorie requirements increase with body size and can be predicted from the formula: Calories for men = 152 ($W_{kg}^{0.73}$). Thus, 25-year-old men of 50, 60, 70 and 80 kg body weight would require 2,600, 3,000, 3,400 and 3,700 calories respectively. Expressed in pounds and percent, men who differ from the reference man in body weight would need:

Weight in pounds	*Percent of standard allowance*
105	80
124	90
143	100
163	110
183	120

These adjustments should be made in terms of desirable body weight if there is clinical evidence or other measurements to indicate significant over- or under-weight.

(2) *Climate*

Calorie intake should be increased 3 percent for every 10° C. decrease in mean annual external temperature from the reference point of 10° C. and vice versa for increases in mean annual temperature.

(3) *Age*

Caloric requirements decrease approximately 5 percent for each decade after age 25.

(4) *Physical Activity*

This is the principal factor causing variation in caloric needs. Caloric levels may have to be increased as much as 50 percent for the heaviest sustained work. This is probably about the maximum (a total of 4,800 calories) for a 65 kg. 25-year-old man. Most often an increase of 20-25 percent (3,800-4,000 calories) will provide adequately for heavy work. On the other hand, truly sedentary 25-year-old men will probably require only about 2,500 calories. The lowest level for extremely sedentary persons is about 1.2 × basal metabolism.

(5) *Other Factors*

There are many factors which may be characteristic of a certain culture such as the use of labor-saving devices, types of physical recreation and perhaps differences in physical efficiency in performing certain tasks which must be considered in estimating requirements.

IV. Clinical Data

1. Suggested Guide for Interpretation of Clinical Findings in Relation to Dietary and Biochemical Data[1]

Nutrient	Clinical sign	Abnormal
Calories:		
Obesity	Skin Thickness:	
	Scapulae	Over 30 mm.
	Lower axillae	Over 25 mm.
	Height-weight tables	Over 10 percent.
Leanness	Skin Thickness:	
	Scapulae	Under 8 mm.
	Lower axillae	Under 8 mm.
	Height-weight tables	Under 10 percent.
Protein	Dependent edema	Over 0 percent under age 50 in absence of beriberi and starvation.
Vitamin A	Follicular keratosis of arms	Over 5 percent in adults.
Thiamine	Absent Achilles tendon reflexes	Over 1 or 2 percent.
Niacin	Tongue lesion more advanced than hypertrophy of papillae at tip.	Over 5 percent.
	Reddened Tongue	Over 1 or 2 percent.
	Pellagrous dermatitis	Over 0 percent.
Riboflavin	Angular stomatitis	Over 5 percent in nondenture wearing population.
	Conjunctival hyperemia (circumcorneal injection).	Over 5 percent.
	Magenta tongue	Over 0 percent.
Ascorbic acid	Red hyperemic gums	Over 5–10 percent (in adults).
	Perifolliculosis	Over 0 percent.

Adapted from Jolliffe, N., "Methods for Evaluation of Nutritional Adequacy and Status", Edited by H. Spector, M. S. Peterson and T. E. Friedemann, Dept. of the Army, Office of the Quartermaster General, p. 201, December 1954.

[1] It should be obvious that these guides are designed for population group surveys since the presence of any one of the clinical findings may have significance in individuals.

Economics and Agriculture

I. Introduction

General information on population, economic conditions, food balances, and the contribution of agriculture to the national economy is needed to make practical recommendations for improvement in the nutritional status. Data on supplies and disappearance of various foods on a per capita basis, if reasonably accurate, indicate in a general way what foods are consumed in relatively large or small quantities, as well as the average level of food consumption. Data on living costs (particularly food prices) and the purchasing power of consumers indicate possibilities and limitations in increasing food purchases as a means of improving diets, especially among low-income groups. Food subsidies may be indicated as a means of improving diets, but the feasibility of such a program would depend on Government policy and the availability of funds. If additional imports of food are required, the availability of foreign exchange and the effect of such a program on the national economy are of vital importance. Available foods from other countries, including surpluses that may be obtained at bargain prices, need to be examined in relation to nutritional requirements for troops and civilians in the country under study.

If additional food is to be grown, more land or fertilizer probably will be needed. Unless more land can be brought under cultivation, increases in the area devoted to food crops will require reductions in land devoted to other crops. Improvements in dietary patterns may be indicated, but recommendations as to what can or ought to be done need to take account of the economic potential as well as the customs and traditions in a region.

The general information and statistics listed in this outline, in so far as possible, should be tabulated and summarized before undertaking the field survey. Supplemental information of a qualitative nature and more details on the areas surveyed should be obtained from informed people at the seat of government, at agricultural colleges, and by inspection of representative farming areas. References on the sources of information should be given. If one is to spend but a few weeks in a country, the time should be spent in survey work that cannot be done elsewhere, rather than in tabulating and summarizing data that are readily available from documentary sources. Among the problems for an agricultural economist to investigate are weakness in present practices and prospects for expanding the production and distribution of foods, especially "protective" foods, such as animal products, fruits and vegetables, by:

More efficient use of land and other resources.
Improving the quality and marketability of farm products.
Reducing food losses, spoilage and waste.
Inter-area surface and air transport of seasonal surpluses.
Providing technical assistance.

The agricultural economist should be prepared to review and evaluate the agricultural and economic aspects of suggestions or recommendations by clinicians for improving the nutritional status.

II. General Population and Economic Aspects

1. Population Characteristics

The objectives of this section are to obtain data on population segments and to establish occupational classification of the population. These data are essential in estimating the nutritional status and food requirements for any area in question.

a. Total Population at Last Census and/or Recent Estimate by State or Province

(1) Population density.
(2) Annual population growth.
(3) Age distribution by groups.
(4) Occupational composition.
(*a*) Industry.—Describe degree of mechanization and modernization. (Purpose is to obtain estimate of energy expenditure.)

	Percent or number	Work hours per week
Agriculture		
Fishing		
Mining		
Manufacturing		
Commerce		
Transportation		
Government and Professional		
Other		

(*b*) Tables of work or labor categories.
(*c*) Refugees and orphans.

b. Principal Religious Faiths and Proportion of Each in Population

c. Educational or Literacy Level Attained by Majority (if indicated, give information separately for different segments of population, for example, farm or rural and urban population.)

Adults	*Youths*
Men	Boys
Women	Girls

d. Agencies and Media for Dissemination of Information

e. Languages and Their Relative Importance

Spoken.
Written.

2. National Income, Prices, Wages

The objective of this section is to obtain actual and relative statistics to indicate trends and the current economic situation.

a. Gross National Product—Total and Per Capita (specify year.)

(1) National income from agriculture, mining, manufacturing.

b. Production

(1) Index numbers of volume
  (*a*) Agriculture.
  (*b*) Mining.
  (*c*) Manufacturing.
(2) Electric power—million kwt.

c. Prices—National and Selected Area Averages

(1) Prices received by producers for major farm products.
(2) Wholesale price of major food and agricultural products.
(3) Retail prices of principal foods and other major cost-of-living items.
(4) Index numbers of prices and living costs in recent years.

d. Wages and Employment

(1) Wage rates and monthly earnings in major occupations.
(2) Unemployment—nature and magnitude.

3. Commerce and Finance

a. Annual Exports and Imports—Quantity and Value by Groups and Major Individual Items

(1) Foods.
(2) Animal feeds.
(3) Industrial crops, such as fibers and tobacco.
(4) Fertilizer.
(5) Other.
(6) Total (value and index number of volume).

b. Foreign Trade—Value by Country and Area

(1) Destination of Exports.
(2) Source of Imports.

c. Foreign Exchange

(1) Dollar rates.
(2) Controls and availability.

d. Credit Facilities and Interest Rates

e. Central Government Finances

(1) Revenue.
(2) Expenditures.
(3) Surplus or deficit.

4. Transportation Facilities and Services

Mileage, general location, and nature of services of:

(1) Roads.
(2) Railroads.
(3) Navigable waterways.
(4) Air Transport.

1. Production

a. Land Utilization

(1) Area.
(2) Agricultural land.
  (*a*) Arable land.
  (*b*) Meadows and pastures.
(3) Potentially productive land.
(4) Agricultural land per capita.
(5) Group distribution by size of farms and farm families.

b. Average Rainfall and Temperatures by Month and by Region

c. Annual Production

(1) Yield per acre and return to producers for major crops: grains, legumes, roots, other vegetables, and fruits.
(2) Population of various types of livestock.
(3) Production of meat, poultry, eggs, milk, cheese, fish and other products.
(4) Plant and animal diseases and pests, their significance and the effectiveness of control measures.
(5) Power—types and relative importance.

d. Types of Production

(1) Extent of large scale production.
(2) Home food production, practice of home gardening, raising poultry and other small animals, large meat animals, fish ponds, etc.
(3) Use of uncultivated or naturally occurring indigenous foods, for example, berries, nuts and fruit.

e. Description of Special Situations and Problems Affecting Agricultural Production

(1) Crop and livestock practices.
(2) Types of implements used.
(3) Land tenure systems and land rents.
(4) Labor supply and employment.
(5) Agricultural credit, taxation.
(6) Government policy and assistance in agriculture.

2. Marketing

a. Organization of Marketing Services

(1) Cooperatives or associations.
(2) City markets (private).
(3) Government markets (controlled).
(4) Transportation of food and feed within the country.

b. Storage in Granaries, Refrigerators, Other Places

(1) Large scale.
(2) Home or farm.

3. Outlook for Production and International Trade in Foodstuffs in an Emergency or Disaster

United States aid programs are designed to strengthen the defense capability as well as the economy of the recipient country. Consequently, an evaluation, at least on a qualitative basis, should be made of the following factors:

(1) What changes could be effected within a year or two so as to increase or maintain food production during an emergency.

(2) What changes might be required under emergency shortages of fertilizer, pesticides, etc.

(3) Vulnerability of food supply.
   (*a*) To local emergencies.
   (*b*) To a general widespread emergency.
   (*c*) To floods, drought, fire or other disasters.

(4) Dependence on imports of food from the U.S.A., from the other free-world countries.

(5) Food surpluses, if any, available for transfer to deficit areas.

4. Food Consumption

The major food groups customarily used in food balance summaries, should be broken down into individual components where these differ markedly in nutritional value. For example, the roots and starchy vegetables should be distinguished from green or leafy vegetables since the former are poor in nutrients and the latter are generally valuable supplementary foods. The reliability of food balance sheets should be checked.

The latest available data on national and regional bases should be compiled on per capita consumption of the principal individual foods and the major groups of food such as:

(1) Grains.
(2) Pulses.
(3) Roots, tubers and starches.
(4) Sugar.
(5) Oilseeds.
(6) Oils and fats.
(7) Vegetables.
(8) Fruits and nuts.
(9) Meats.
(10) Fish.
(11) Milk products.
(12) Eggs.
(13) Others.

5. Development of Education and Research in Agriculture

It is the purpose of this section to evaluate the effects of educational programs in agriculture on the national nutritional program. This includes international assistance and the United States Mutual Assistance programs. It is well to:

(1) Describe the national and local plans, programs or measures for increasing food production.

(2) Give estimates, if feasible, as to the effects of such programs on food production.

(3) Educational and research programs—list or describe.

(*a*) Agricultural colleges.

(*b*) Experiment stations.

(*c*) Extension programs.

(*d*) Student exchange programs.

(*e*) Demonstrations and large scale projects by national and international groups on methods of conservation, use of modern machinery, etc.

(4) Describe other measures, past and present, that have been taken to increase food production.

(5) Evaluation of efficiency of agricultural methods.

Food Technology

I. Introduction

Information in the area of food technology is needed in order to provide a basis for practical recommendations for improving nutritional status. Firsthand information is needed to ascertain local needs and desires, as well as what food and nutrition programs are feasible in the country under study.

The food technologist will work closely with the nutritionist and agricultural economist in obtaining and evaluating data of mutual interest.

Food processing, preservation, and proper storage may offer means by which the quantity and quality of foods may be increased materially.

Agricultural readjustments may lead to better nutrition from domestic crops or from imported foods obtained through an increase in international trade in agricultural products.

Changes in the technical assistance program may be needed to facilitate improvements in agricultural practices and the development of processing techniques.

United States agricultural surpluses may be able to supplement native food supplies provided they can be incorporated into acceptable foods and the facilities for processing can be made available.

Efforts should be made to get detailed information on past and present nutrition programs. A number of programs for nutritional improvement are being conducted at the present time by national and international agencies. In most instances the proposed products have not been widely used because of either poor acceptability or technical difficulties.

Data should be obtained on nutritionally important products to allow evaluation of practicability under the following criteria: (1) the product must be suitable to the soil and climate of the region; (2) the processing methods must be consistent with the available technical skills; (3) the form in which the food is to be eaten must be acceptable to the people; (4) the cost must be low.

The general procedure to be followed by the food technologist, on reaching the country to be surveyed, will be to consult first with the American Agricultural Attaché and various officials of the International Cooperation Administration in the country under study. Through these men the availability of reliable statistics can be learned and contact made with specialists in the local Department of Agriculture.

The specialists to be interviewed should include experts in agricultural production, home economics, food processing, and nutrition. Installations to be visited should include experiment stations, agricultural colleges, representative food processing plants, and demonstrations of new agricultural techniques.

In addition to the above, key men in the Armed Forces, including American advisors, if any, should be interviewed with respect to rations and food services and arrangements made to visit typical military establishments and facilities for food handling.

Variations in food practices in different areas of the country should be observed by visiting markets, restaurants, and homes.

II. Areas of Interest

1. Food and Nutrition Services of the Armed Forces

(See corresponding section under "Dietary Studies")

The food technologist will supplement the work of the nutritionist in obtaining information on food and nutrition services in the Armed Forces. Geographical and seasonal differences in rations may be related to local variations in food production and lack of adequate transport or storage. An extension of the use of troops in producing and processing foods may be a means of correcting nutritional deficiencies. The production and processing techniques employed by the Quartermaster may be capable of improvement. Differences in rations between officers and men may indicate supplementary foods that are acceptable to native tastes. The costs of those foods, which might make an important contribution to the diet if supplied to men as well as officers, should be determined. The amounts of foods issued to the men may be partially determined by the extent to which foods are bartered or sold to obtain other commodities. This practice may make it difficult to substitute one item for another in the ration.

2. Regulations and Controls Affecting Foods

The food technologist will work with the agricultural economist in obtaining information on regulations and controls affecting foods. The possible effect of these controls in preventing or facilitating agricultural readjustments and changes in the quality of the food supply will be considered. The need for additional regulations and inspections, for example, covering a minimum degree of milling or requiring fortification of certain foods, should be explored.

3. Agricultural Production

(See Chapter 7, section III)

Data obtained by the agricultural economist on the production, storage, transport, and marketing of farm products will be examined by the food technologist, on the basis of his own observations, to determine the technological reasons for waste and spoilage; also for evaluating the present and potential supply of farm products that may be available to food processing plants.

4. Consumption and Evaluation

(See Chapter 5, section II, 4)

In order to relate the per capita consumption of major foods to nutritional status it is necessary to take into account major differences in important population groups due to climate, economic status, racial backgrounds and religious customs.

a. Proximate Composition and Nutritive Value of National Food Supply

b. Composition, Nutritive Value, Relative Importance, and Cost of Individual Foods As Purchased

The individual foods available in markets and stores should be identified by name and description and considerable supplementary information obtained covering species and variety of crop, method of handling

or processing, type of container, price per unit, local or seasonal availability, universality and frequency of consumption, etc.

c. Recipes and Descriptions of Prepared Foods

Commonly used foods should be named and described with supporting information on method of cooking, ingredients, amount eaten, and frequency of use, losses in preparation and cooking, kitchen equipment and fuel used.

d. Basic Diets

Efforts should be made to estimate the additional cost of providing an adequate diet to important groups by a comparison of actual diets with the national average diet and a minimum adequate diet.

(1) Composition and cost of national average diet.
(2) Composition and cost of diet of poorer segments in the population.
(3) Composition and cost of minimum adequate diet.

5. Preservation

Preservation is here distinguished from processing in that it is likely to be cruder and carried out on a small scale.

a. Large Scale Preservation

Refrigeration, sun-drying, and salting.

b. Home or Farm Preservation

Refrigeration, pasteurization, sun-drying, milling, fermenting, salting, others.

6. Processing and Manufacture

Processing and preserving foods provide means of utilizing seasonal crops on a year-round basis in a form capable of more economical storage and transport to various markets. Through the elimination of seasonal gluts and shortages the effective food supply is increased. Data on food processing plants should include localities, products, capacities, availability of raw materials, processing methods, equipment, and market outlets. Information should be obtained on the following:

(1) Canning and can manufacture.
(2) Baking.
(3) Meat packing and use of by-products.
(4) Fermentation (sauerkraut, yogurt, etc.).
(5) Salt manufacture, mined or sea salt.
(6) Iodization of table salt.
(7) Vegetable oil—Cottonseed, soybean, mustard, corn, etc. Use in margarine manufacture.
(8) Vitamin manufacture.
(9) Enrichment.
(10) Milling.
    (*a*) Scope of operation—private, group, or association.
    (*b*) Use of byproducts such as bran from rice.
    (*c*) Regional customs in processing, such as parboiling of rice and use of calcium carbonate in milling of rice.
    (*d*) Customary degree of milling or percent reduction.

(11) Breweries—proportion of cereal crop used.

(12) Sugar manufacture.

(13) Other food manufactures.

(14) Discussion of special situations and problems such as sanitation, availability of foods for processing, processing costs, losses, containers, etc.

7. Regulations and Controls Affecting Foods

(Source, type and availability of food as determined by government action.)

a. Food Allowances

Items covered for general use and for special groups.

b. Rationing

Items and quantities.

c. Enrichment or Fortification of Foods

d. Inspection and Enforcement of Regulations Relating to Foods

The foregoing outline on Food Technology aspects is tentative and will necessarily have to be revised in accordance with the situation existing in the country under survey. It is likely that all the desired information will not be obtainable either because of inadequate records or due to the shortness of time allotted to the survey.

Supplemental Data To Be Collected

The collection of general population statistics is covered in another section of this Manual. However, other types of facts and figures may be quite helpful in interpreting nutrition problems, if such information is available. *Special note must always be taken of the extent to which these data can be considered accurate and reliable.*

It is the responsibility of the Director, or Team Leader, to distribute work assignments among the members of the Nutrition Survey Team so that as far as possible information on the following subjects may be obtained.

I. Civilian Health Statistics

(Indicate trends if data are available)

(1) Crude death rates.
(2) Crude birth rates.
(3) Infant death rates.
(4) Birth weights and lengths.
(5) Infant feeding practices.
(6) Growth rates of school children.
(7) Maternal death rates.
(8) Principal causes of death.
(9) Average life expectancy of males and of females.
(10) Incidence of disease:
Tuberculosis, pneumonia, diarrhea, and dysentery, malaria, parasitic infestation, etc.
(11) Medical care.
(*a*) Number and training of physicians, nutritionists, nurses, midwives.
(*b*) Number, size and description of hospital and laboratory facilities.
(12) Nutrition services and nutrition research facilities:
Major laboratories, personnel, program, needs.
(13) Preventive medicine and public health services:
Names, training of personnel, scope of program, administrative placement in government, needs.
(14) Medical education facilities:
Schools, student enrollment, requirements for practice.
(15) Sanitation practices and problems.

II. Military Health

(1) Medical standards for military acceptance.
(2) Induction screening:
Number and causes of rejections; height-weight-age data.

(3) Noneffective rates:
General and by cause where possible.
(4) Medical, hospital and laboratory services.
(5) Nutrition and/or dietetic services.
(6) Numbers and causes of military medical discharges.
(7) Sanitation (general problems not covered by dietary studies).

III. Educational, Religious, and Cultural Aspects

Any factors which would bear on food consumption, eating habits, food taboos, etc.

Laboratory Equipment and Supplies[1]

I. Electrical Equipment and Supplies[2]

1. Centrifuges and Accessories

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Centrifuge, laboratory, size 1, model SBV "International", 115 volts, 50-60 cycle AC with steel guard bowl, auto. transformer controller, tachometer, timer, integral sub-base mounting and casters. International Equipment Corp. (IEC) (Cat. No. 5449)	1	Each	$669.00	$669.00
(2) Head, 8-place, (Cat. No. 240)	1	Each	30.00	30.00
(3) Shields, Metal, 10 ml., with cushions. IEC No. 356	24	Each	1.25	30.00
(4) Shields, Metal, 50 ml., with cushions. IEC No. 320	8	Each	1.90	15.20
(5) Ring, Trunnion, Brass for Centrifuge cup, 50 ml. size, IEC No. 325	8	Each	1.30	10.40
(6) Carrier Trunnion, Brass for Centrifuge cup, triple carrier, IEC No. 355	8	Each	2.24	17.92
(7) Cap reducing aluminum Centrifuge tube, IEC No. 312	8	Each	.30	2.40
(8) Cushion, rubber for 50 ml. Centrifuge cup, IEC No. 571	12	Each	.18	2.16
(9) Cushion, rubber for 15 ml. Centrifuge cup, IEC No. 570	12	Each	.09	1.08
(10) Tube, centrifuge, resistant glass, conical, beaded rim, plain, Corning No. 8060, 15 ml. capacity	180	Each	.19	34.20
(11) Tube, centrifuge, resistant glass, conical, beaded rim, plain, Corning No. 8060, 50 ml.	36	Each	.40	14.40
(12) Tube, centrifuge, pyrex, conical, heavy duty, plain, $\overline{\$}$ 24/40 stopper, 40 ml.	3	Dozen	26.76	80.28
(13) Tube, centrifuge, resistant glass, conical, beaded rim, graduated, Corning No. 8080, 15 ml.	72	Each	.88	63.36
(14) Tubes, hematocrit, blood vol. index, Wintrobe Kimble No. 46748	120	Each	1.15	138.00
(15) Centrifuge, clinical, with 6-place head for 15 ml. tubes, complete with head, 6 metal shields and 6 rubber cushions. IEC No. 458	2	Each	127.00	254.00

[1] Laboratory Equipment—In numerous instances possible sources of supply are indicated merely to clarify specifications. These items are available from other sources. In requesting supplies for the Nutrition Survey Teams from Interdepartmental Committee on Nutrition for National Defense, it is only necessary to quote the section and item number, e.g., tubes, centrifuge, pyrex, 40 ml., would be ordered as I–1–12.

[2] All electrical equipment based on 60 cycle, 110 volt. If 50 cycle or other voltage is to be used, specifications for equipment must be revised or adequate transformers ordered.

I. Electrical Equipment and Supplies—Continued

2. pH Meter

	Quantity	Unit of issue	Estimated unit price	Total price
(16) pH Meter, portable model N–1 complete with batteries, glass electrode, reference electrode, electrode holder, 50 ml. beaker, pt. of pH 7.00 buffer solution, 100 ml. KCl solution, KCl crystals and cleaning tissues	1	Each	$290.00	$290.00
(17) Electrode, glass, 0 to 11 pH Beckman No. 4990–80	1	Each	17.50	17.50
(18) Electrode, reference, calomel, Beckman No. 4970	1	Each	14.00	14.00
(19) Tubes, electrometer, style K, Beckman No. 12576	2	Each	7.50	15.00
(20) Batteries, B–15V., Eveready No. 417, Beckman No. 12572	8	Each	.50	4.00
(21) Batteries, B–22—½ v., Everedy No. 470. Beckman No. 12571	4	Each	.10	.40
(22) Batteries, D–1½ v., Everedy No. 950. Beckman No. 4999	2	Dozen	.10	.20

3. Photofluorometer

(23) Photofluorometer, Coleman [3] model 12C, (Cat. No. 12–005) complete with lamp and transformer; one dozen cuvettes, (Cat. No. 12–190) without filters. Operating instructions. 60 cycle	1	Each	$450.00	$450.00
(24) Cover, instrument, (Cat. No. 6–215)	1	Each	3.50	3.50
(25) Cuvettes, 19 x 105 mm., round, (Cat. No. 12–190)	12	Dozen	11.00	132.00
(26) Filter, primary, thiamine B_1, (Cat. No. 12–221)	1	Each	11.00	11.00
(27) Filter, primary, thiamine, low concentration, (Cat. No. 12–225)	1	Each	10.00	10.00
(28) Filter, primary, riboflavin (B_2), (Cat. No. 12–222)	1	Each	12.00	12.00
(29) Filter, secondary, thiamine (B_1), (Cat. No. 14–211)	1	Each	12.00	12.00
(30) Filter, secondary, riboflavin (B_2), (Cat. No.14–212)	1	Each	8.00	8.00
(31) Electronipak, spare amplifier, complete	1	Each	100.00	100.00
(32) Coleman manual of clinical methods. (For the Coleman photofluorometer model 12C)	1	Each	15.00	15.00
(33) Graph paper, 50 sheets/pad (Cat. No. 14–321)	6	Pad	3.00	18.00
(34) Operating directions (extra)	1	Each	2.00	2.00

4. Spectrophotometer Equipment

(35) Spectrophotometer, Coleman [4] Jr., model 6A, complete with battery and charger, (6–053), (Cat. No. 6–009)	1	Each	$380.00	$380.00
(36) Adapter for cuvettes, 10 x 75 mm., round (Cat. No. 6–108)	2	Each	9.00	18.00
(37) Adapter for cuvettes, 19 x 150 mm., round (Cat. No. 6–102)	2	Each	9.00	18.00
(38) Battery, Willard storage [4] 6 volt, dry	1	Each	30.00	30.00

[3] If current is 50 cycle, then a specially built photofluorometer 12C is required since the standard model, 60 cycle, will not operate on 50 cycle current. Price on this instrument is $415.00.

[4] Acid for battery to be packed in separate glass containers with absorbent insulation in a fiber carton and labeled in accordance with Export Air Shipment requirements.

	Quantity	Unit of issue	Estimated unit price	Total price
(39) Cuvette, 10 x 75 mm., round, selected (Cat. No. 6–310)	12	Dozen	$10.00	$120.00
(40) Cuvette, 19 x 150 mm., round, selected (Cat. No. 6–304–B)	24	Dozen	11.00	264.00
(41) Filter, didymium, calibrating Standard (Cat. No. 6–400)	1	Each	16.00	16.00
(42) Instrument cover for spectrophotometer, Coleman Jr. model 6A, (Cat. No. 6–215)	1	Each	3.50	3.50
(43) Operating directions for spectrophotometer, Coleman Jr., model 6A, (Cat. No. 6–900)	1	Each	2.00	2.00
(44) Galvanometer assembly for spectrophotometer, Coleman Jr., model 6A	1	Each	35.00	35.00
(45) Galvanometer lamp, 2 per set, (Cat. No. 6-502)	2	Set	2.50	5.00
(46) Exciter Lamp, (Cat. No. 6–500)	2	Each	6.00	12.00
5. Others				
(47) Blendor, Waring, electric for 115 volt, 25–60 cycle AC-DC, 11,000 r.p.m., at one pint water load, stainless steel knives and propeller blades, Aminco No. 4–3320	2	Each	$32.42	$64.84
(48) Cord, Electrical, heavy duty, 50 ft.	1	Each	1.50	1.50
(49) Demineralizer, portable, 5 gal./hr., (Deeminizer)	1	Each	39.50	39.50
(50) Deeminite unit, for use with above item, 6 refills/carton. Ion exchange resin cartridge	2	Carton	11.50	23.00
(51) Water still, electric, stainless steel, 1½ gallon per hour, heavy duty, complete with automatic cutoff, for 230 volts, Barnstead Model No. 32022	1	Each	300.00	300.00
(52) Heating unit for use with above item (4 units required for use with still)	4	Each	15.40	61.60
(53) Generator, portable, (Cat. No. 3 W A) 120 volts, AC-DC, 3,000 Watt, with pneumatic wheels	1	Each	575.00	575.00
(54) Hot plate, Lindberg, size 12 x 20 inches, 2600 wattage, type H–2	1	Each	102.50	102.50
(55) Electric hot plate, 3 speed, low-medium-high, 7″ diameter, 660 watts Chromalox-ROPH 7066. 115-120 volts	3	Each	13.77	41.31
(56) Oven, sterilizer 30″ x 18″ x 24″ with automatic thermostat, Despatch model 287-A (complete with accessories)	1	Each	218.00	218.00
(57) Plugs, electrical, adapter 3 prong to 2 prong	12	Each	Lot	.10
(58) Plugs, electrical, adapter U.S. 2 prong to continental round	12	Each	.10	1.20
(59) Shaker, utility, with two adjuster bars, without Kahn rack, Clay Adams No. A–2305	1	Each	93.00	93.00
(60) Sterilizer, instrument, electric, inside dimensions, 13″ x 5″ x 4″, Castle No. 5–413	1	Each	64.79	64.79
(61) Stepdown transformer, (Am. Inst. Co.) 220 volt. 50 cycle to 115 volt. 50 cycle: 150 watts	4	Each	13.00	52.00
(62) 500 watts	1	Each	23.00	23.00
(63) 2,400 watts 2.5 KVA	2	Each	54.00	108.00

I. Electrical Equipment and Supplies—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(64) 250 watts	2	Each	$15.00	$30.00
(65) 1,000 watts	1	Each	42.75	42.75
(66) 1,500 watts	1	Each	40.75	40.75
(67) Test volt ammeter, model 630, Triplett	1	Each	44.50	44.50
(68) Transformer, variable voltage regulator No. 4–801	1	Each	25.00	25.00
(69) Vacuum pump, rotary, power driven, 110 volt., 60 cycle AC, 250-watt	1	Each	81.90	81.90

II. Balances, Scales and Accessories

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Balance, analytical with beam 0 to 1 gm. with magnetic damping attachment. Single vane type A. H. Thomas No. 1853	1	Each	$286.00	$286.00
(2) Balance, weights analytical 5 mg. to 200 gms. National Bureau of Standards, class S, Thomas No. 19734	1	Set	48.80	48.80
(3) Balance, triple beam, stainless steel, 111 gm. capacity, complete with 3 rider weights	2	Each	24.75	49.50
(4) Balance, triple beam, Ohaus type, with attached weights, capacity 600 gms.	2	Each	15.45	30.90
(5) Extra weight for above balance, 500 gm.	1	Each	.76	.76
(6) Balance, solution, metric, capacity, large double pan each 9″ diameter, 5 kilogram capacity, including set of brass weights 1 gr. to 2 kilograms; No. S–3395	1	Each	60.00	60.00
(7) Scales, Fairbanks-Morse Model No. 12006, platform scale beam construction, size 11″ x 11″, capacity 300 lbs., ¼ lb. intervals. (Overall height—13½″)	3	Each	62.00	186.00
(8) Scale, Fairbanks-Morse code No. 1124, portable platform, beam 100 x ½ lbs., capacity 1,000 lbs., platform 18″ x 27″	1	Each	66.10	66.10
Special packing charges				15.00

III. Glassware

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Beakers, low form with spout, pyrex, Corning 1,000: 50 ml.	24	Each	$0.21	$5.04
(2) 100 ml.	48	Each	.21	10.08
(3) 250 ml.	48	Each	.22	10.56
(4) 400 ml.	24	Each	.24	5.76
(5) 600 ml.	24	Each	.32	7.68
(6) 1,000 ml.	8	Each	.74	5.92
(7) 2,000 ml.	4	Each	1.42	5.68
(8) Bottles, prescription amber glass with screw cap, 2-oz. Armstrong CTA	3	Gross	6.00	18.00

	Quantity	Unit of issue	Estimated unit price	Total price
(9) Bottles, clear glass square, wide mouth, screw caps, Armstrong No. 49–906: 4 oz.	4	Dozen	$ .40	$1.60
(10) 8 oz.	4	Dozen	.52	2.08
(11) Bottle, glass stopper, reagent, narrow mouth, pyrex, flat head 4,000 ml. capacity, ℔ Stopper No. 45	2	Each	3.33	6.66
(12) Burette, dispensing, resistance glass with stopcocks, 10 ml. capacity in 1/20 ml. Subdivisions Kimble No. 17030	2	Each	3.50	7.00
(13) Burette, dispensing, resistance glass with stopcock, 25 ml. capacity in 1/10 ml. Subdivisions Kimble No. 17030	2	Each	3.77	7.54
(14) Burette, dispensing, resistance glass with stopcock, 50 ml. capacity in 1/10 ml. Subdivisions Kimble No. 17030	2	Each	4.61	9.22
(15) Carboys, bottles, boxes with cork cushions, 5-gallon capacity (Cat. No. 2–950 Fisher)	2	Each	7.30	14.60
(16) Cylinder, graduated, with spout, single graduations, 100 ml., 1 ml. Subdivisions Kimble No. 20025 12/Case	1	Case	36.57	36.57
(17) Ditto except 250 ml., 2 ml. subdivisions Kimble No. 20025	6	Each	3.40	20.40
(18) Ditto except 500 ml., 5 ml. subdivisions Kimble No. 20025	6	Each	4.66	27.96
(19) Ditto except 1,000 ml., 10 ml. subdivisions Kimble No. 20025	6	Each	6.52	39.12
(20) Ditto except 2,000 ml., 20 ml. subdivisions Kimble No. 20025	2	Each	10.26	20.52
(21) Cylinder, graduated, mixing, for stopper ℔ No. 13, 25 ml. Kimble No. 20036	6	Each	6.60	39.60
(22) Stopper ℔ No. 13 for use with item above Kimble No. 42000	6	Each	.44	2.64
(23) Cylinder, graduated Beaker, double spout, double scale, Corning No. 6480: 125 ml.	6	Each	2.54	15.24
(24) 500 ml.	6	Each	4.24	25.44
(25) 1,000 ml.	2	Each	5.73	11.46
(26) Crucible, Gooch, 35 ml. diameter, high form, glazed throughout, without cover, Coors porcelain No. 290	12	Each	.89	10.68
(27) Desiccator, with cover, pyrex, large size, with ℔ sleeve. Inside diameter ground flange 200 mm. Corning No. 3120	1	Each	22.80	22.80
(28) Dish, evaporating, regular style, with lip, Coors porcelain No. 430, 110 mm. diam., height 43 mm., capacity 200 ml. No. 4	4	Each	.93	3.72
(29) Dropper, medicine, straight with bulb	24	Each	.02	.48
(30) Distilling apparatus (Graham) with condenser 19/38, 1000 ml. (Corning 3360)	2	Each	16.78	33.56
(31) Flask, Erlenmeyer, pyrex, narrow mouth, 50 ml. approximate stopper No. 1, Corning No. 4980	96	Each	.26	24.96
(32) Flask, Erlenmeyer, pyrex, narrow mouth, 125 ml. approximate stopper No. 4, Corning No. 4980	24	Each	.26	6.24
(33) Flask, Erlenmeyer, pyrex, narrow mouth, 1,000 ml., approximate stopper No. 9, Corning No. 4980	4	Each	.55	2.20

	Quantity	Unit of issue	Estimated unit price	Total price
(34) Flask, filtering, pyrex, heavy wall, tabulation, 1,000 ml. capacity stopper No. 8, Corning No. 5340	2	Each	$1.53	$3.06
(35) Flask, volumetric, pyrex, Corning No. 5640, 10 ml. with ⅓ stopper No. 9	48	Each	1.80	86.40
(36) Flask, volumetric, pyrex, Corning No. 5640, 25 ml. with ⅓ stopper No. 9	48	Each	1.95	93.60
(37) Flask, volumetric, pyrex, Corning No. 5640, 100 ml. with ⅓ stopper No. 13	72	Each	1.72	123.84
(38) Flask, volumetric, pyrex, Corning No. 5640, 250 ml. with ⅓ stopper No. 16	4	Each	2.27	9.08
(39) Flask, volumetric, pyrex, Corning No. 5640, 500 ml. with ⅓ stopper No. 16	4	Each	2.50	10.00
(40) Flask, volumetric, pyrex, Corning No. 5640, 1,000 ml. with ⅓ stopper No. 22	4	Each	3.22	12.88
(41) Funnels fluted, accurate 60° long stem, pyrex, Corning No. 6160, 65 mm. inside diameter, 150 mm. length of stem	24	Each	.33	7.92
(42) Funnels fluted, accurate 60° long stem, pyrex, Corning No. 6160, 100 mm. inside diameter, 150 mm. length of stem	6	Each	.45	2.70
(43) Hydrometer, specific gravity, range 1.000 to 1.070 length 150 mm	2	Each	2.95	5.84
(44) Hydrometer, specific gravity, range 1.000 to 1.225 length 165 mm	2	Each	1.75	3.50
(45) Hydrometer, syringe for lead acid storage batteries, specific gravity range 1.130 to 1.310, Willard No. 16791	1	Each	.81	.81
(46) Mortar, glass with pestle and lip, 8-oz. capacity, 4⅝" outside diameter	2	Each	1.32	2.64
(47) Pipettes, serological "Lifetime Red" graduations, Corning No. 7083, 5 ml. capacity in 1/10 graduation	24	Each	.98	23.52
(48) Pipettes, serological "Lifetime Red" graduations, Corning No. 7083, 10 ml. capacity in 1/10 graduation	72	Each	1.45	104.40
(49) Pipettes, serological, pyrex, Corning No. 7080 1/10 ml. capacity in 1/100 graduation	12	Each	.85	10.20
(50) Pipettes, serological, pyrex, Corning No. 7080 2/10 ml. capacity in 1/100 graduation	12	Each	.98	11.76
(51) Pipettes, serological, pyrex, Corning No. 7080 1 ml. capacity in 1/100 graduation	12	Each	1.20	14.40
(52) Pipette, automatic for acid, 1 ml. with rubber bulb; No. J–2121	4	Each	4.25	17.00
(53) Pipettes, pyrex, Corning No. 7100: 1 ml. transfer	12	Each	1.00	12.00
(54) 2 ml. transfer	6	Each	.90	5.40
(55) 3 ml. transfer	12	Each	.89	10.60
(56) 4 ml. transfer	12	Each	1.24	14.88
(57) 5 ml. transfer	12	Each	1.00	12.00
(58) 10 ml. transfer	12	Each	1.10	13.20
(59) 20 ml. transfer	12	Each	.90	10.80
(60) Pipette, constant, volume syringe type, Krogh-Keys, No. 69290: 1 ml.	6	Each	10.50	63.00
(61) 2 ml.	6	Each	10.50	63.00
(62) 5 ml.	12	Each	12.30	147.60
(63) 10 ml.	6	Each	14.65	87.90
(64) 20 ml.	2	Each	14.65	29.30

	Quantity	Unit of issue	Estimated unit price	Total price
(65) Plate, desiccator, glazed on one side, without feet, with numerous small perforations, Coors porcelain No. 590 size 4, 190 mm. diameter, 1 mm. perforation	1	Each	$4.46	$4.46
(66) Rod, stirring, soft glass, 5 mm. diameter x 150 mm. long	12	Length	.02	.24
(67) Rod, stirring, soft glass, 10 mm. diameter x 300 mm. long	12	Length	.10	1.20
(68) Spatula porcelain spoon glazed on one end—Coors No. 650, 123 mm. length, size No. 1a	6	Each	.70	4.20
(69) Specific gravity bottle, conical shape, 25 ml. with thermometer, $\overline{\$}$ 10/18 Joint; Kimble No. 15123 range +12° to +38° in 0.2 intervals	2	Each	6.06	12.12
(70) Syringe, hypodermic slip, resistance glass, 5 ml. capacity in 1/5 graduation Aloe No. A–636B	24	Each	1.25	30.00
(71) Syringe, hypodermic slip, resistance glass, 10 ml. capacity in 1/5 graduation, Aloe No. A–636C	36	Each	1.76	63.36
(72) Syringe, hypodermic slip, resistance glass, 20 ml. capacity in 1 ml. graduation, Aloe No. A–636D	144	Each	2.06	296.64
(73) Syringe, tuberculin, Luer slip, resistance glass, Aloe No. 77710, 1 ml. capacity in 1/100 ml. subdivision	12	Each	2.75	33.00
(74) Syringe, tuberculin, Luer slip, resistance glass, Aloe No. 77710, 2 ml. capacity in 1/50 ml. subdivision	12	Each	1.73	20.76
(75) Thermometer, general laboratory, total immersion minus 10° to plus 110° centigrade, Kimble No. 43528	2	Each	1.12	2.24
(76) Thermometer, general laboratory, total immersion 10° to plus 205° centigrade, Kimble No. 43508	2	Each	1.20	2.40
(77) Thermometer, general laboratory, total immersion minus 10° to plus 360° centigrade, Kimble No. 43518	2	Each	1.16	2.32
(78) Thermometer, clinical, centigrade 34°–42° oral, without case, class B, triangle lens type	3	Each	.52	1.56
(79) Tubes, connecting, Y shape glass, Kimble No. 45030 $\frac{1}{4}$″ diameter, 1$\frac{1}{2}$″ arm length	6	Each	.20	1.20
(80) Tubes, test with solid penny-head $\overline{\$}$ 13 stopper 16 x 150 mm. complete	6	Dozen	31.32	187.92
(81) Tubes, test, with black plastic screw caps 16 x 125 mm. Kimble No. 45066	2	Case	34.22	68.44
(82) Tubes, base exchange, absorption Hennessey clear pyrex glass, 50 ml. No. 18–234	4	Dozen	21.00	84.00
(83) Tubes, specimen vacutainers 20 ml. 6$\frac{1}{2}$″ x $\frac{5}{8}$″ (BD No. 3208) 240/pkg.	2	Package	15.78	31.56
(84) Tubes, vacutainer with anticoagulants, 6 mg. ammonium oxalate 4 mg. potassium oxalate, 7 ml. BD, No. 3204 KNH, 300/pkg	2	Package	14.80	29.60
(85) Tubing, soft glass, standard wall, 5-foot length 4 mm. outside diameter	6	Length	.03	.18
(86) Tubing, soft glass, standard wall, 5-foot length 5 mm. outside diameter	6	Length	.05	.30

III. Glassware—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(87) Tubing, soft glass, standard wall, 5-foot length 6 mm. outside diameter	6	Length	$.05	$.30
(88) Tubing, soft glass, standard wall, 5-foot length 8 mm. outside diameter	6	Length	.08	.48
(89) Vacutainer, needles, 1½″ BD No. 3200N	5	Dozen	1.65	8.25
(90) Vacutainers, adapter, holder to Luer needle, BD No. 3200A	2	Dozen	1.65	3.30
(91) Vacutainers, holder, plastic, BD No. 3200H	2	Dozen	1.87	3.74

IV. Miscellaneous Equipment and Supplies

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Adhesive plaster, surgical, 3″ x 5 yards	36	Roll	$.40	$14.40
(2) Applicator, wood, 6½″ length x 1/12″ diameter, Aloe No. 2770, 6 gross per package	24	Package	.37	8.88
(3) Asbestos, American, selected fiber, acid-washed and ignited (for Gooch crucibles)	¼ lb.	2	.50	1.00
(4) Bag, physicians, Boston, size 17″, black, smooth, topgrain cowhide Coleman No. 70238	2	Each	27.00	54.00
(5) Basin, wash, stainless steel, 13⅝″ diameter x 3⅜″ depth	4	Each	3.81	15.24
(6) Basket, test tube, galvanized wire, 6 x 6 x 6″ No. 78352	12	Each	1.46	17.52
(7) Beakers, polyethylene, 1,000 ml	4	Each	4.60	18.40
(8) Bottle, Boston, round, polyethylene, with screw cap, 32 oz	140	Each	.46	64.40
(9) Bottle, Boston, round, polyethylene, with screw cap, 16 oz	36	Each	.24	8.64
(10) Bottle, Boston, round, polyethylene, with screw cap, 8 oz	4	Each	.14	.56
(11) Bottle, polyethylene, wide mouth, with screw cap, 32 oz	24	Each	1.25	29.90
(12) Brush, Burette, bristle 5″ length, tapered ⅝″ to 1″. Set in wire handle rubber-tipped end	2	Each	.11	.22
(13) Brush, test tube, bristle, set in wire handle, bristle ½″ x 3½″ overall length 8″	12	Each	.09	1.08
(14) Brush, test tube, bristle set in wire handle, bristle 1½″ x 3½″. Overall length 10″, dome end	12	Each	.11	1.32
(15) Brush, beaker, set in ¾″ diameter wood handle, bristle part including tuft measuring 5″; 3″ diameter; overall length 16″	24	Each	.23	5.52
(16) Burner, 42 mm. grid diameter, natural gas, 1,200 Btu	4	Each	3.25	13.00
(17) Burner, Micro, 6 mm. tube diameter, 135 mm. inlet tube length natural gas, 1,200 Btu	4	Each	.66	2.64
(18) Burner, gas, blast, Universal Jr.	1	Each	18.50	18.50
(19) Charts, color, test for color blindness, Ishikara	1	Each	12.50	12.50
(20) Chart, visual sign, (illiterate type), No. C3058	1	Each	.60	.60

	Quantity	Unit of issue	Estimated unit price	Total price
(21) Clamp, extension, rubber sleeve, large grip up to 2½″ diameter, 9″ length. Castaloy No. 5-733	6	Each	$.79	$4.74
(22) Clamp, Pinchcock, for closing tubing up to ¼″ wall. Castaloy No. 5–849A	12	Each	.36	4.32
(23) Clamps, test tube, Stoddards, 120 mm. length, Chicago Apparatus No. 10640	6	Each	.15	.90
(24) Clamps, hosecocks, with brackets. Castaloy No. 5–847	12	Each	.63	7.56
(25) Clamp, Burette support, double only, hold any size burette from micro to 100 ml. capacity. Alumaloy No. 1056	2	Each	2.00	4.00
(26) Clamps, versatile, 3 prong rubber sleeves, 10½″ long, grip from 1/16″ to 3¼″ diameter. Castaloy No. 5–742	6	Each	1.13	6.78
(27) Clamps, combination, utility and burette, small, hold up to 1½″ diameter, rubber sleeve, Alumaloy No. 1025	12	Each	.86	10.32
(28) Cooker, pressure, 4-qt	2	Each	12.95	25.90
(29) Cork, borers, brass, with ramrod, 5 to 19 mm. 12 to set, Fisher No. 7–845	1	Each	2.90	2.90
(30) Cotton, absorbent, sterile USP	5	1-lb roll	.77	3.85
(31) Counters, hand tally, Veeder, tallies up to 10,000	2	Each	3.70	7.40
(32) Cups, paper "Dixie", nonwax, 8 oz., 50/box	40	Box	.64	25.34
(33) Cups, paper, 16-oz. (milk shake type) Stott No. 116, 100/pkg	1	Package	2.17	2.17
(34) Lids for item above, Stott No. 516	1	Package	.54	.54
(35) Depressor, tongue, wood, ¾″ wide x 6″ long, 500/pkg	10	Package	.71	7.10
(36) Files, hand, American standard square, second cut, 6″ bastard	6	Each	.38	2.28
(37) Filter paper, circles, Whatman's No. 1, 11 cm	12	Package	.28	3.36
(38) Filter paper, circles, Whatman's No. 1, 18.5 cm	3	Package	.85	2.55
(39) Filter paper, Whatman's No. 40, quantitative, 11 cm	10	Package	1.84	18.40
(40) Filter paper, Whatman's No. 42, quantitative, 11 cm	10	Package	1.75	17.50
(41) Food jar, insulated metal case with insulated cover, self-locking handle, glass fiber, capacity 1 gallon Beth-mont No. 8204000	4	Each	29.23	116.92
(42) Forceps, dressing, thumb, straight, fine serrated tips, 4½″ long, stainless steel, Aloe No. H–2613	3	Each	.74	2.22
(43) Fork, tuning, 128 VIB/SEC No. 85C–3135	2	Each	2.60	5.20
(44) Gauze, surgical, 36″ wide, medium mesh, 100 yards to bolt	1	Bolt	7.15	7.15
(45) Glass wool, fibrous glass, fiber diameter 0.0002 to 0.0003″ sheets of 8-oz. rolls	2	Roll	.50	1.00
(46) Gloves, rubber, autopsy, heavy, size 7½	4	Pair	.31	1.24
(47) Gauze, iron with asbestos center, 5″ x 5″, Fisher No. 15–590	6	Each	.17	1.02
(48) Hammer, reflex testing, Taylor regular size 8″, No. A–129	2	Each	.87	1.74
(49) Kit, first aid, medium size for 25–50 persons, J. & J. insurance case No. 8–5578	1	Each	21.93	21.93

	Quantity	Unit of issue	Estimated unit price	Total price
(50) Lantern, battery operated, Delta Powerlite, 800-foot beam at front, Floodlight at top. Noland No. A–1530	2	Each	$5.60	$11.20
(51) Lantern batteries for use with item above	2	Each	.98	1.96
(52) Lubricant, (silicon) stopcock grease, M. P. 400° F	3	Tube	1.25	3.75
(53) Matches, safety, wood, 40 to a box	50	Box	.01	.50
(54) Needle, hypodermic, syringe, Luer slip, long bevel, 20 gage, 1½″, Aloe No. A–642	18	Box	1.24	22.32
(55) Needle, hypodermic, short bevel, 18 gage, 5″, Empire	3	Dozen	2.50	7.50
(56) Needle, hypodermic, short bevel, 16 gage, 3½″, Empire	1	Dozen	2.25	2.25
(57) Needle, hypodermic, syringe, Luer slip, long bevel, 22 gage, 1¼″, Aloe No. A–642	4	Box	1.72	6.88
(58) Needle, hypodermic, syringe, Luer slip, long bevel, 23 gage, ⅝″, Aloe No. A–642	4	Box	1.08	4.32
(59) Nitrogen, type 1—oil free, 230 cu. ft. per cylinder	1		3.45	3.45
(60) Nitrogen, cylinder only, 230 cu. ft. size	1	Each	40.00	40.00
(61) Nitrogen tank regulator, two stage reduction, Thorpe tube, complete with adapter to fit ¼″ tubing National No. 764–T1	1	Each	51.50	51.50
(62) Oil, Cenco vacuum pump, heavy weight, Cenco No. 93055B	4	1-qt Can	.65	2.60
(63) Ophthalmoscope-Otoscope combination set, consisting of No. 110 Ophthalmoscope and No. 216 Otoscope with 5 specula, with large handle, extra bulbs, in leatherette case, Welch-Allyn No. 983	1	Each	64.80	64.80
(64) (Ophthalmoscope-Otoscope), batteries for use with Item above	4	Lot	.50	2.00
(65) Pad, gauze, 2″ x 2″, 12 ply, J. & J., 100/pkg.	20	Package	.50	10.00
(66) Paper, Test, pH Hydrion, range 2 to 10 pH in dispenser holding 2—15-foot rolls, with color chart, No. 64080	6	Each	1.50	9.00
(67) Pail, polyethylene, with lid, 11 quart size	2	Each	3.80	7.60
(68) Pencils, glass writing, "Nonrun," heat resisting, red	48	Each	.12	5.76
(69) Pipette jar, polyethylene, 5″ x 18″, Nalgene No. 1242, Size C	2	Each	9.20	18.40
(70) (Pump), coupling, filter pump, smooth, ¼ IP. No. 9–980	1	Each	.60	.60
(71) (Pump), coupling, filter pump, smooth ⅜″ IP. No. 9–980	1	Each	.60	.60
(72) (Pump), coupling, filter pump, threaded with ⅜″ IP female thread and a ¼″ male thread, No. 9–978	2	Each	.90	1.80
(73) Rack, test tube, Wasserman, nickel plated brass 13 mm. tube size, 40 tube capacity. Aloe No. 14610	6	Each	3.50	21.00
(74) Rack, test tube, Wasserman, stainless steel, 20 mm. tube capacity, 4″ x ½″ size tubes, No. 14–805	6	Each	2.20	13.20
(75) Rule, slide pocket, plastic, 5″. K & E No. 4161–1	2	Each	5.63	11.26
(76) Rule, slide, polyphase, 10″. K & E No. 4053–3	4	Each	12.15	48.60

IV. Miscellaneous Equipment and Supplies—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(77) Scissors, operating, straight, one sharp point, one blunt point, 4½″. Aloe No. S–104C	2	Each	$1.80	$3.60
(78) Shears, office, steel, 6″	2	Each	.70	1.40
(79) Shears, office, steel, 9″	2	Each	.97	1.94
(80) Skinfold calipers with calibrating weight	2	Each	35.00	70.00
(81) Snow Man (dry ice manufacturing machine) capacity 30Z pellet 3″ diameter by 1″ thick, portable, complete	1	Each	85.00	85.00
(82) Spatula, flexible steel, wood handle, 200 mm. blade length	4	Each	.47	1.88
(83) Spatula, flexible steel, 100 mm. blade length	10	Each	.36	3.60
(84) Sphygmomanometer, mercurial portable type with standard arm band, complete calibrated to 206 mm. No. A–1	2	Each	24.50	48.50
(85) Stand, support, rectangular base, size 6½″ x 11″ (extra large) Fisher No. 14–670	3	Each	1.84	5.52
(86) Stethoscope, Rieger-Bowles, combination type	3	Each	6.86	20.58
(87) Stone, sharpening 2″ x 6″	1	Each	.40	.40
(88) Stopper, cork XXXX quality, regular length, No. 0	200	Each	.01	2.00
(89) Stopper, cork XXXX quality, regular length, No. 5	200	Each	.01	2.00
(90) Stopper, cork XXXX quality, regular length, No. 6	200	Each	.01	2.00
(91) Stopper, cork XXXX quality, regular length, No. 7	200	Each	.01	2.00
(92) Stopper, rubber, solid, No. 1 (55/lb.)	3	Pound	.69	2.07
(93) Stopper, rubber, solid, No. 4 (33/lb.)	1	Pound	.69	.69
(94) Stopper, rubber, solid, No. 5 (28/lb.)	1	Pound	.79	.79
(95) Stopper, rubber, solid, No. 6 (21/lb.)	1	Pound	.88	.88
(96) Stove, gasoline burner, 2-burner type, heavy duty, Coleman No. 413E	2	Each	17.95	35.90
(97) Support, test tube, Kahn, neoprene, coated, 30-tube capacity No. 14530	4	Each	2.50	10.00
(98) Support, test tube, rack, ⅝″ diameter of holes, 48 holes, nickel plated brass, Fisher No. 14804–5	3	Each	1.72	5.16
(99) Tape, masking, manila, crepe, moisture proof, 1″ wide, 60-yard roll	4	Roll	.60	2.40
(99a) Tape, masking, manila, crepe, moisture proof, only ½″ wide, 60-yard roll	4	Roll	.36	1.44
(100) Tape, measuring, spring wound return, metal, in case, length 6-feet or 2 meters, graduated in cm. and inches. No. 52610	3	Each	1.30	3.90
(101) Timer, stopwatch, interval Kodak, 0 to 60 seconds, in ½ second divisions	1	Each	9.00	9.00
(102) Timer, interval, spring operated, 1 to 120 minute, Cenco No. 73411	3	Each	8.92	26.76
(103) Tissue, paper, cleaning	48	Box	.22	10.56
(104) Tongs, crucible, steel, double bent, 9″ length, Aloe No. 80010	3	Each	1.51	4.53
(105) Towel, hand	36	Each	.20	7.20
(106) Triangles, wooden (for height meas.,) ¾″ plywood	3	Each		
(107) Tripod, iron, threaded legs, single ring, 9″ height, 3⅛″ inside diameter 5″ outside diameter	3	Each	.45	1.35
(108) Tube, connecting, brass Y-shape, ¼″. No. 80680	6	Each	.50	3.00

IV. Miscellaneous Equipment and Supplies—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(109) Tubing, rubber, pure gum, amber, bore 3/16", wall 3/32"	24 ft.	Feet	$.16	$3.84
(110) Tubing, rubber, pure gum, amber, bore 3/16", wall 1/16"	24 ft.	Feet	.10	2.40
(111) Tubing, rubber, pure gum, black, bore 1/4", wall 3/32"	12 ft.	Feet	.15	1.80
(112) Tubing, rubber, pure gum, amber, bore 3/8", wall 3/32"	12 ft.	Feet	.14	1.68
(113) Tubing, rubber, pure gum, black, bore 3/16", wall 3/64"	12 ft.	Feet	.10	1.20
(114) Twine, Jute, 1/2-pound balls	2	Ball	.29	.58
(115) Water bath, Army Medical School model, 8" diameter x 4" deep, with tripod legs 9" long	3	Each	8.75	26.25
(116) Pump, filter, Richards, 7" length, IPS thread connection 3/8", with 1/8" IPS threaded air inlet tube, complete w/ threaded and friction couplings. No. 43310 and No. 43550	2	Each	5.00	10.00
(117) Tubing, rubber, pressure, bore 1/4", wall 3/16"	12	Foot	.40	4.80
TOOL KIT TO INCLUDE THE FOLLOWING				
(118) Hammer, claw, nail, 1 pound	1	Each	$.90	$.90
(119) Screwdriver, ordinary, 3" length, 3/16" blade	1	Each	.11	.11
(120) Screwdriver, ordinary, 6" length, 5/16" blade	1	Each	.16	.16
(121) Screwdriver, Phillips, No. 1251, 3" blade	1	Each	.25	.25
(122) Wrench, monkey, 10", 1 3/4" opening of jaw, 41–W–2342	1	Each	1.65	1.65
(123) Pliers, combination slip-joint 6" (mechanics)	1	Each	.28	.28
(124) Pliers, side cutting, lap-joint, 6 1/2" (linemans)	1	Each	1.10	1.10
(125) Tape, electrical, clear scotch, 1/2" x 66-foot roll, No. 27	2	Roll	1.75	3.50
(126) Knife, paring, 3"	2	Each	.56	1.12
(127) Solder, solid wire, no flux type, 1/8" diameter for electrical appliance and radio repair work "bit". 1 pound per spool	5	Lot	6.25	6.25
(128) Soldering iron to be used with above item	1	Each	3.50	3.50
(129) Brace and bits, for wood and metal	1	Set	3.00	3.00
(130) Carpenter hand saw, 20" long	1	Each	2.50	2.50

V. Chemicals

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Acetic, anhydride, reagent [1]	5	1-pound bottle	$1.07	$5.35
(2) Acetone, reagent ACS [3]	10	1-pound bottle	.66	6.60

See footnotes on Page 153.

V. Chemicals—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(3) Acid, acetic, glacial, reagent [1]	4	1-pound bottle	$.97	$3.88
(4) Acid, hydrochloric, reagent [1]	8	1-pound bottle	.46	3.68
(5) Acid, metaphosphoric, glacial, pellets, reagent [2]	6	1-pound bottle	3.48	20.88
(6) Acid, oxalic, crystals, reagent [2]	4	1-pound bottle	1.28	5.12
(7) Acid, potassium phthalate, Bureau of Standards 51–L–50–100	2	60-gram bottle	4.72	9.44
(8) Acid, sulfuric, reagent [1]	36	1-pound bottle	.65	23.40
(9) Alcohol, ethyl, 95 percent [3]	18	1-quart can	.26	9.36
(10) Ammonium hydroxide, reagent 10 percent solution specific gravity 0.95 [1]	2	1-pound bottle	2.31	4.62
(11) Ammonium oxalate, crystals reagent	1	1-pound bottle	1.95	1.95
(12) Ammonium sulfate, granular C. P.	4	1-pound bottle	.80	3.20
(13) Antimony trichloride, C. P. [2]	4	¼-pound bottle	2.26	9.04
(14) Bromcresol green	2	1-gram bottle	2.90	5.80
(15) Isobutyl alcohol, C. P. [3]	40	1-quart cans	2.25	90.00
(16) Charcoal, activated Norite powdered	2	1-pound	1.65	3.30
(17) Chloroform, reagent [3]	9	1-pound bottle	.52	4.68
(18) Citric acid, powder, reagent monohydrate [2]	2	1-pound bottle	1.32	2.64
(19) Cupric sulfate crystals, reagent (copper sulfate) ($CuSO_4$ $5H_2O$) ACS, fine or powdered	25	1-pound bottle	1.68	42.00
(20) 2, 4-Dinitrophenylhydrazine, M. P. 199–200°, E. K. No. 1866 [6]	10	25-gram bottle	1.10	11.10
(21) 2, 6-dichloro benzenone indophenol, sodium salt	25	Gram	Lot	21.00
(22) Detergent, Alconox	6	3-pound package	2.05	12.30
(23) Ether, anhydrous, C. P., ACS [3]	4	1-pound can	.90	3.60
(24) Ether, petroleum, benzine special, C. P. boiling point 20–40° C. [3]	5	1-pound	1.10	5.50
(25) Ferric ammonium sulfate $Fe\,NH_4(SO_4)_2 \cdot 12\,H_2O$, C. P. 51–L–50–100	1	1-pound bottle	1.48	1.48
(26) Heparin, at least 60 Toronto units per 0.1 gram	3	1-gram bottle	6.35	19.05
(27) Hydrogen peroxide 30 percent reagent hyperoxide [1]	1	1-pound bottle	4.18	4.18
(28) Maleic acid	2	1-pound bottle	4.30	8.60

See footnotes on Page 153.

V. Chemicals—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(29) Metaphen, tincture, 1:200, tinted	1	Pint	$2.17	$2.17
(30) Alcohol, methyl (methanol) special reagent	2	1-pound bottle	.66	1.32
(31) Paraffin, 50–52° C., white, U.S.P.	2	1-pound cake	.45	.90
(32) Picric acid recrystallized, C. P. [5]	2	1-pound bottle	4.67	9.34
(33) Potassium chloride, reagent	15	1-pound bottle	1.10	16.50
(33a) Potassium, dihydrogen phosphate (dibasic) secondary crystals, reagent	2	1-pound bottle	1.34	2.68
(34) Potassium, phosphate (monobasic), primary, crystals, reagent	2	1-pound bottle	1.68	3.36
(35) Potassium ferricyanide, crystals, reagent	2	1-pound bottle	2.75	5.50
(36) Potassium ferrocyanide, crystals, reagent	2	1-pound bottle	1.85	3.70
(37) Potassium hydroxide pellets, reagent [2]	2	1-pound bottle	1.44	2.82
(38) Potassium iodide, crystals, reagent	1	1-pound bottle	4.00	4.00
(39) Potassium oxalate, crystals, reagent	2	1-pound bottle	2.73	5.46
(40) Potassium permanganate, crystals, reagent	1	1-pound bottle	1.41	1.41
(41) Potassium sodium tartrate crystals, reagent	2	1-pound bottle	1.68	3.36
(42) Petrolatum liquid, heavy U.S.P.	1	Quart	.75	.75
(43) p-Toluene sulfonic acid (25 gm.) [2]	4	25 gram bottle	1.20	4.80
(44) Quinine sulfate	2	1-ounce bottle	1.63	3.26
(45) Sodium acetate, crystals	6	1-pound bottle	1.37	8.22
(46) Sodium bicarbonate $NaHCO_3$, C. P.	2	1-pound bottle	.70	1.40
(47) Sodium chloride granular reagent	2	1-pound bottle	.61	1.22
(48) Sodium bichromate (sodium dichromate), reagent	4	1-pound bottle	1.32	5.28
(49) Sodium hydroxide, pellets reagent [2]	10	1-pound bottle	1.10	11.00
(50) Sodium hydrosulfite, powder, pure, (low in iron) [2]	2	¼-pound bottle	2.10	4.20
(51) Sodium sulfate anhydrous powder, reagent	12	1-pound bottle	.95	11.40
(52) Sodium sulfate, anhydrous granular, reagent	2	1-pound bottle	.97	1.94
(53) Sodium tungstate crystals, reagent	4	¼-pound bottle	2.88	11.52

See footnotes on Page 153.

V. Chemicals—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(54) Thiochrome decalso permutit-T: for thiamine determination, 50–80 mesh (Fisher T–97)	4	5-pound bottle	$26.25	$105.00
(55) Thiourea	2	1-pound bottle	2.52	5.04
(56) Toluene [3]	3	1-pound can	.75	2.25
(57) Trichloracetic acid, crystal, reagent [1]	1	1-pound bottle	4.65	4.65
(58) Tris (hydroxymethyl) Amino methane	2	100-gram bottle	3.10	6.20
(59) DL—Tryptophane [4]	2	1-pound bottle	.....	.....
(60) Water soluble tablets of thiamine hydrochloride [4]	1000	Tablets 5 milligram each	.....	.....
(61) Water soluble tablets of riboflavin [4]	1000	Tablets 5 milligram each	.....	.....
(62) Water soluble tablets of nicotinamide [4]	1000	Tablets 50 milligram each	.....	.....
(63) Water soluble tablets of ascorbic acid [4]	1000	Tablets 100 milligram	.....	.....
(64) Sterns (canned heat)	24	Cans	.15	3.60
STANDARDS				
(65) Ascorbic acid	4	25-gram bottle	$1.50	$6.00
(66) Beta carotene, 10 mg. vial	3	1-ampule	3.68	11.04
(67) Cholesterol U.S.P.	1	100-gram bottle	2.25	2.25
(68) Creatinine zinc chloride	2	10-gram vial	2.10	4.20
(69) N'Methylnicotinamide standard [4]	5	50-milligram vial	.....	.....
(70) Riboflavin standard	2	25-gram bottle	4.35	8.70
(71) Fluorescein disodium salt, reference standard (Eastman organic chemicals No. 735) (To be used for chemical purposes only)	2	25-gram	3.25	6.50
(72) Thiamine hydrochloride	2	10-gram bottle	2.00	4.00
(73) Vitamin A standard	25	Gram	5.75	5.75
(74) Xanthurenic acid [4]	3	60-milligram vial	.....	.....

[1] White Label—"Corrosive liquid or acid." Each to be packed in 1 pint glass bottle, maximum, plus absorbent cushioning and put in metal container and each container to be packed in individual strong shipping box.

[2] Orange Label—"Corrosive when wet." Individual containers of not more than 5-pounds each with a total maximum 25-pounds in one package, packed in absorbent material for air shipment.

[3] Red Label—"Flammable liquids." Must be in 1 pint glass or 1 quart metal maximum plus absorbent cushioning, each unit to be packed in individual strong shipping container.

[4] To be supplied by manufacturer.

[5] For air shipment, must contain over 10 percent moisture, labeled "Class A explosive," packed as per white label.

[6] Flammable solid label, packed same as per red label.

VI. Office Supplies

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Calculator, Monroe Ln Model, 160–X (item No. 54–M–16206) (a)	1	Each	$250.00	$250.00
(2) Clinical cards, abbreviated	7,000			
(3) Folders, file, wallet type, 1¾″ expansion with tie, full gusset, 9½″ x 11¾″	25	Each	.16	4.00
(4) Kodachrome clinical slide boxes, metal/100 slides, Brumberger No. 1050	3	Each	1.53	4.59
(5) Kodaslide viewer, Sawyer's Bi-Lens 35	2	Each	3.96	7.92
(6) Ledgers, blank book, 8½″ x 10½″, record ruled, (not indexed)	18	Each	.70	12.60
(7) McBee cards, duplicates with carbon inserts—500 cards per package	5	Package	150.00 per 1000	375.00
(8) McBee card savers No. K4S, packed 10 pads per package	1	Package	2.80	2.80
(9) McBee keysorts, No. 5005	4	Each	3.50	14.00
(10) McBee punches, No. 5201	3	Each	6.60	19.80
(11) Notebooks, leatherette, 6¾″ x 3¾″, flexible	8	Each	1.50	12.00
(12) Fillers, lined for above	20	Package	.13	2.60
(13) Pads, 8″ x 10½″ ruled	18	Each	.15	2.70
(14) Paper, graph, semilogarithmic sheets, 8½″ x 11″, Plate 7″ x 10″, 3-cycle x 10 divisions to the inch, 5th and 10th lines progressively accented, green, Dietzgen 340–L310	50	Sheet	.02	1.00
(15) Paper, typewriting bond, 25 percent rag, white, 8″ x 10½″	2	Package	1.22	2.44
(16) Paper, carbon, black, lightweight, medium finish 8″ x 11″	2	Box	.71	1.42
(17) Pencils No. 2, medium soft	3	Dozen	.15	.45
(18) Pencils No. 3, medium hard	3	Dozen	.16	.48
(19) Sharpener, pencil, office	2	Each	1.01	2.02
(20) Pencils, blue and red	2	Dozen	.37	.74
(21) Pens, ball point, Venus type, blue-black ink	24	Each	.28	6.72
(22) Refill, ball point pen, blue-black ink, to fit Venus model	24	Each	.12	2.88
(23) Paper, typewriting, manifold carbon sets (Letterex) 8″ x 10½″, white	1	Box	1.84	1.84
(24) Numbering machine, automatic, 6-wheel, 5-movement	1	Each	13.25	13.25
(25) Stamp, dating, rubber-band, metal frame, 4 revolving bands, month, day and year	2	Each	.24	.48
(26) Pad, ink, No. 4, 2⅝″ x 1⅝″ for above item	2	Each	.66	1.32
(26a) Ink, stamp pad for felt pads, 2-oz. bottle, blue	2	Bottle	.10	.20
(27) Dietary survey forms ICNND No. 1	50	Each		
(28) Dietary survey forms ICNND No. 2	50	Each		
(28) Dietary survey forms ICNND No. 3	50	Each		
(29) Dietary survey forms ICNND No. 4	50	Each		
(30) Dietary survey forms ICNND No. 5	50	Each		
(31) Dietary survey forms ICNND No. 6 [1]	50	Each		
(32) Dietary survey forms ICNND No. 7 [1]	50	Each		

[1] For use in civilian survey.

VII. Books

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Albritton, E.C., ed. Standard values in nutrition and metabolism. Philadelphia, Saunders, 1954	1	Book	$6.50	$6.50
(2) Association of Official Agricultural Chemists. Official methods of analysis of the Association of Official Agricultural Chemists. 8th ed. Washington, D.C., The Association, 1955	1	Book	12.50	12.50
(3) Assocation of Vitamin Chemists. Methods of vitamin assay 2d. rev. ed. N.Y., Interscience, 1951	1	Book	6.00	6.00
(4) Chatfield, C. Food composition tables—minerals and vitamins—for International use. Rome, Italy, Food and Agriculture Organization of the United Nations, March 1954. (FAO nutritional studies, No. 11)				
Available free to Government agencies from: Foreign Agricultural Service United States Department of Agriculture Washington 25, D.C.				
Others can purchase from: N.Y., Columbia University Press, $1.00	2	Book	1.00	2.00
(5) Cochran, W. G. Sampling Techniques. N.Y., Wiley, 1953	1	Book	6.50	6.50
(6) Consolazio, C.F., Johnson, R.E., and Marek, E. Metabolic methods. St. Louis, Mo., Mosby, 1951	1	Book	5.00	5.00
(7) Harrison, T.R., ed. Principles of internal medicine. 2d. ed. N.Y., Blakiston, McGraw-Hill, 1954	1	Set	21.00	21.00
(8) Hawk, P.B., Oser, B.L., and Summerson, W.H. Practical physiological chemistry. 13th ed. N.Y., Blackiston, McGraw-Hill, 1954	1	Book	12.00	12.00
(9) Hill, A.B. Principles of medical statistics. 6th ed. rev. and enl. N.Y., Oxford University Press, 1955	1	Book	4.00	4.00
(10) Jolliffe, N., Tisdell, F. and Cannon, P., eds., Clinical Nutrition, New York, Harpers & Brothers, 1950. (This book is out of print)				
(11) Lange, N.A., comp. Handbook of chemistry. 9th ed. Sandusky, Ohio, Handbook publishers, 1956	1	Book	8.50	8.50
(12) Lorie, J.H., and Roberts, H.V. Basic methods of marketing research. N.Y., McGraw-Hill, 1951	1	Book	6.00	6.00
(13) McLester, J.S., and Darby, W.J., Nutrition and diet in health and disease. 6th ed. Philadelphia, Saunders, 1952	1	Book	10.00	10.00
(14) Manson-Bahr, P.H. Manson's tropical diseases. 14th ed. Baltimore, Williams and Wilkins, 1954	1	Book	12.50	12.50
(15) The Merck index of chemicals and drugs. 6th ed. Rahway, N.J., Merck, 1952	1	Book	8.00	8.00
(16) Rand Corporation. A million random digits with 100,000 normal deviates. Glencoe, Ill., The Free Press, 1955	1	Book	10.00	10.00
(17) Sunderman, F.W., and Boerner, F. Normal values in clinical medicine. Philadelphia, Saunders, 1949	1	Book	15.00	15.00
(18) Todd, J.C., Sanford, A.H., and Wells, B.B. Clinical diagnosis by laboratory methods. 12th. ed. Philadelphia, Saunders, 1953	1	Book	8.50	8.50

VII. Books—Continued

	Quantity	Unit of issue	Estimated unit price	Total price
(19) United States Department of Agriculture. Composition of foods used in Far Eastern countries. Washington, D.C., United States Government Printing Office, March 1952. (Agriculture Handbook No. 34)	2	Book	$.30	$.60
(20) United States Department of Agriculture. Composition of foods raw,—processed,—prepared. Washington, D.C., United States Government Printing Office, June 1950. (Agriculture Handbook No. 8)	2	Book	.55	1.10
(21) United States Department of Agriculture. Table of food composition for the Armed Forces. Washington, D.C., United States Government Printing Office, January 1951				
This is also available to Government agencies from: Office of the Adjutant General. Washington 25, D.C. Issued as (United States Department of the Army. Technical bulletin QM 58, October 1951)	2	Book		
(22) Wohl, M.G., and Goodhart, R.S., eds. Modern nutrition in health and disease. Philadelphia, Lea & Febiger, 1955	1	Book	18.50	18.50
(23) Yates, F. Sampling methods for censuses and surveys. 2d. ed. rev. and enl. London, C. Griffin, 1953	1	Book	5.32	5.32

VIII. Medicinals for Team Use

	Quantity	Unit of issue	Estimated unit price	Total price
(1) Acetylsal cylic acid tablets, 100 to bottle	3	Bottle	$0.27	$0.81
(2) Aluminum hydroxide tablets	200	Tablets	.75	1.50
(3) Caffeine and sodium benzoate injectable 2 ml. 250 mg./CC, 12 to box	2	Boxes	3.50	7.00
(4) Camphorated opium tincture, 4-oz. bottle	2	Bottle	.60	1.20
(5) Chloroquine phosphate tablets, 0.5 gm.	100	Tablets	5.35	5.35
(6) Compound dimethyl phthalate stick (612) 1-oz.	10	Stick	.59	5.90
(7) D.D.T. aerosol bombs	4	Bomb	1.59	6.36
(8) D.D.T. dusting powder 1½ oz.	4	Cans	.25	1.00
(9) Dimenhydrinate tablets 50 mg	200	Tablets	4.41	8.82
(10) Glyceryl trinitrate tablets (sublingual) 0.6 mg.	100	Tablets	.45	.45
(11) Meperidine hydrochloride injection 50 mg./ml.	5	Ampules	1.76	1.76
(12) Neosynephrine nose drops, 15 ml. bottles, .25 percent	4	Bottle	.70	2.80
(13) Primaquine phosphate	100	Tablets	3.25	3.25
(14) Propantheline bromide tablets, 15 mg.	100	Tablets	4.55	4.55
(15) Secobarbital sodium capsules, 100 mg.	100	Capsules	2.70	2.70
(16) Sulfadiazine tablets 0.5 gm.	100	Tablets	1.69	1.69
(17) Tetracycline capsules (Tetracyn) 0.25 gm. per capsule	300	Capsules	17.00	51.00
(18) Zinc undecylenate ointment 1 oz.	4	Tubes	.54	2.16
(19) Zinc undecylenate powder 1½ oz.	4	Cans	.54	2.16
(20) Water purification tablets (tetraglycine hydroperiodide each tablet liberates 8.0 mg. iodine)	1,000	Tablets		

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